PROGRESS REPORT

of the

ANIMAL DISEASE AND PARASITE RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1967, and June 30, 1968. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

UNITED STATES DEPARTMENT OF AGRICULTURE

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INTRODUCTION

The Animal Disease and Parasite Research Division administers a national program of basic and applied research on diseases of cattle, poultry, swine, sheep, horses, and fur-bearing animals. The Division consists of three large laboratories and 11 smaller, specialized laboratories. The large ones are the Beltsville Parasitological Laboratory, the National Animal Disease Laboratory, Ames, Iowa, and the Plum Island Animal Disease Laboratory at Greenport, New York. The research at these locations covers, respectively, animal parasites, animal diseases existing in the United States, and foreign animal diseases. The smaller, specialized laboratories are located as follows:

Southeast Poultry Research Laboratory, Athens, Georgia
Regional Parasite Research Laboratory, Auburn, Alabama,
with substation at Experiment, Georgia
Endoparasite Vector Pioneering Research Laboratory,
Pullman, Washington
Toxicological Research Laboratory, Kerrville, Texas
Southwestern Veterinary Toxicology and Livestock Insects
Research Laboratory, College Station, Texas
Animal Disease Research Laboratory, Denver, Colorado
Poisonous Plants Research Laboratory, Logan, Utah
Parasite Research Laboratory, Albuquerque, New Mexico
Parasite Research Laboratory, Las Cruces, New Mexico
Swine Parasite Research Laboratory, Tifton, Georgia
Cooperative Research at the East African Veterinary
Research Organization, Kabete, Kikuyu, Kenya, East Africa

In addition, the Division engages in other research involving 35 cooperative projects and research contracts at various universities and State Experiment Stations. The Division's research program is coordinated by the Office of the Director, located at Beltsville, Maryland.

The Animal Disease and Parasite Research Division has contributed many significant research findings aimed at reducing the heavy losses to the livestock industry resulting from animal diseases. Several of these research discoveries have accounted for savings to the livestock industry in excess of the total cost of animal disease research in the U.S. Department of Agriculture since the inception of the Bureau of Animal Industry in 1887. Among these discoveries are the isolation and description of the genus of bacteria known as Salmonella; the role of arthropod vectors in spreading infectious diseases; the cause of hog cholera and the development of the first immunization procedure for this disease; the first successful treatment for hookworms in animals and man; the development of strain 19 vaccine to prevent brucellosis, and the discovery of the cause of hyperkeratosis in cattle. Some of the more recent accomplishments are:

Biochemical effect of nitrates and other nitrogenous compounds in sheep. Acute poisoning was induced in sheep with ammonium chloride, ammonium sulfate, and a mixture of ammonium chloride, ammonium carbonate, and ammonium phosphate. Toxicity was due to the ammonium ion, since signs of poisoning were the same regardless of the anion.

In addition to the lowering of blood pH and greatly elevated blood sugar levels reported last year, serum potassium and nonprotein nitrogen levels were markedly increased during ammonium ion poisoning. The total amino acids of the nonprotein nitrogen fraction of the blood were also increased. The effects upon blood sugar levels are due to an adrenergic response, or an interference with sugar metabolism, or both. The elevated blood potassium levels are due to massive cellular breakdown during poisoning. The increase in amino acids indicates an overloading of the urea cycle.

Carriers of foot-and-mouth disease (FMD). After recovery from FMD, many cattle become carriers of the virus. The virus is located in the throat and nasal areas of carriers, and may be found in fluids or cells from these areas for many months after infection. Vaccinated animals may become carriers following exposure to infection, and many of them do not show any clinical signs of infection. Sheep and goats also can become carriers, but the carrier state has not been found in swine. These findings are of great importance in regard to the importation of animals from countries where the disease is sometimes present, and in relation to pathogenesis and control.

Foot-and-mouth disease vaccine (FMDV) for swine. An inactivated, trivalent FMDV, using an oil adjuvant, produced an enduring level of immunity in swine for at least six months. The posterior side of the ear was chosen as the preferable area for vaccination by the subcutaneous route; this choice resolves the possibility of carcass blemish should there be any question of a local reaction at the inoculation site.

Cell-free foot-and-mouth disease virus-ribonucleic acid synthesis. An enzyme-ribonucleic acid (RNA) complex has been found in FMDV-infected cells that is not detected in normal, uninfected cells. This complex is the functional unit that synthesizes as many as 10,000 viral RNA molecules per infected cell. The enzyme-RNA complex has been taken out of the cell and partially purified in an active state. It synthesizes three classes of viral-specific RNA in the test tube. This enzyme-RNA complex can be inhibited by an antibody found in serum of animals recovering from FMD. The complex was further subfractionated into two functionally different enzyme-RNA complexes. One synthesizes viral RNA, and the other synthesizes double-stranded RNA. The latter RNA is not found in uninfected cells, and is a potent agent in the production of interferon in animals. Interferon is a highly efficient inhibitor of virus replication.

The results of a complement fixation (CF) test for equine infectious anemia revealed that the initial CF antibody response varied in its time of appearance but usually occurred during or slightly after the first fever elevation. After attaining its highest level, the titer slowly fell over a variable period of time. Occurrence of a second or subsequent febrile episodes did not produce a recall response. The CF antibody titer continued to fall and became undetectable after several weeks to several months.

Bovine pulmonary emphysema was experimentally produced following feeding of an amino-acid, DL-tryptophan. The onset, course, clinical signs, and pulmonary lesions were strikingly similar to those features observed in the spontaneously occurring disease of cattle. The natural disease is often observed following a change of forage, while the experimental disease was induced by a sudden "overload" of a naturally-occurring dietary constituent.

Juvenile bovine lymphosarcoma. Studies of two naturally-occurring cases in animals less than six weeks of age indicate possible *in utero* spread. A syncytial virus isolated from adult bovine lymphosarcoma was injected into the fetuses of pregnant cows to determine if the condition could be reproduced with the agent. The results should indicate whether the dam can or cannot transmit the syncytial virus to the fetus, and also remain a carrier of the virus.

Hamster paratuberculosis. Histopathologic examination of hamsters inoculated with *Mycobacterium paratuberculosis* revealed extensive invasion and pathologic alterations in the lymphatic tissue. The lesions were extensive throughout the body, and persisted for over a year. The results of the study indicate possibilities of using laboratory animals in preliminary evaluation of vaccines, and therapeutic studies with paratuberculosis.

Mycotic abortion experimentally induced. Spores from a strain of *Aspergillus fumigatus*, originally isolated from a naturally-occurring case of mycotic abortion in a cow, produced abortion when injected into pregnant ewes. A definite dose-response has been established, and lesions typical of naturally-occurring field cases have been reproduced. Fungal infection preferentially involved the placenta and fetal tissues while sparing the maternal tissues. This involvement emphasized the necessity of examining the placenta when attempting to diagnose the cause of abortion. Elucidation of natural routes of infection are being sought for this important cause of bovine reproductive failure.

Endocrine factors play a major role in the pathogenesis of milk fever. Studies of the pathogenesis of postparturient paresis in dairy cattle have revealed: (1) the severe hypocalcemia produces a diabetic condition since there is an inability to release insulin in hypocalcemic states, (2) the diabetic state is further complicated by an increased activity of

the pituitary-adrenal axis, and (3) both of these factors contribute to the depression observed in the latter stage of this condition.

Preliminary results indicate that embryonating chicken eggs (intravascular route of inoculation) are equal to sheep inoculation for detecting bluetongue virus from field-collected cattle and sheep blood. Each assay system, sheep versus eggs, missed one different sample from sheep and four from cattle. Sheep inoculations are estimated to cost $100, while the use of eggs as the virus isolation system is somewhat under $10 per test.

Bluetongue virus may cause deformities in calves born to dams infected in the 1st third of their gestation period. A heifer experimentally infected with bluetongue virus, via bites of bluetongue-infected Culicoides variipennis, at 90 days of its gestation period had a live calf at parturition. The calf had improper dentition and a misalignment of its lower jaw. This work supports previous results obtained from two cattle infected under natural conditions in which an abortion occurred, as well as a deformity and dwarfism in the live calf. The work emphasizes the need for further research on bluetongue in pregnant cattle since it has already been shown that cattle may act as reservoirs of bluetongue virus.

Experimental use of feed supplements to prevent livestock poisoning from poisonous plants has given very encouraging results in two years of range experimental studies. If such a procedure can be successful, it may be a method to prevent the loss of thousands of range livestock each year from poisonous plants.

The heat treatment of chicken hatching eggs to inactivate Mycoplasma gallisepticum. In studies employing 2,000 egg loads, no cultures of M. gallisepticum were obtained from sample trays of eggs from infected hens when the eggs reach 114 to 115°F. during 13- to 15-hour treatments as compared to the proved infected control eggs. Data from control eggs heated during these studies showed that loss of hatchability was not extreme, although more severe heat treatments reduced hatchability to below 50 percent. It is suggested that this procedure may be used to break the egg transmission cycle of M. gallisepticum in primary breeding stock if a moderate reduction of hatchability could be tolerated.

New strain of infectious bronchitis virus identified. A new strain of infectious bronchitis virus isolated in Georgia has been identified as being serologically distinct from previously-reported types of infectious bronchitis virus. Preliminary cross-challenge data also indicate that this virus is immunologically distinct. The virus causes typical signs of infectious bronchitis in chickens and in 10-day-old chicken embryos and is ether sensitive.

New drugs eliminate immature liver flukes from sheep. Several drugs effectively eliminate adult liver flukes but most are ineffective against immature flukes except at dosage levels toxic to sheep. In recent investigations using both naturally- and experimentally-infected ewes, Tremerad, Bayer 9015A, and sulfoxide of bithionol eliminated more than 95 percent of the flukes, six weeks of age and older, with little, if any, toxicity to the sheep. Since most of the damage in acute fascioliasis is caused by immature flukes migrating through the liver tissue, these drugs are potentially of great value to sheepmen in fluke-infested areas.

New intermediate hosts for swine nematodes. Examination of beetles captured in southwest Georgia resulted in the addition of 72 species to the list of known intermediate hosts of swine nematodes. Thirteen species of dung beetles, not previously recognized as intermediate hosts, harbored infective stages of the thick stomach worms of swine, Physocephalus sexalatus and Ascarops strongylina. Four other species of beetles, not usually associated with dung, were found infected with the same nematodes. Five new records were established for dung beetles serving as intermediate hosts of the swine gullet worm, Congylonema pulchrum.

Antioxidant used as preservative in feed found to reduce nematode larval development. Ethoxyquin (Santoquin) reduced developmental potential of parasitic larvae when added to the feed supplement or when added to feces from infected calves.

Cytochemical methods used to detect enzymes in coccidia. The application of cytochemical methods to the study of Eimeria stiedae, the cause of coccidiosis of the liver in rabbits, has revealed the presence and distribution of a number of important enzymes in these parasitic protozoa, including acid and alkaline phosphatase, carboxylic ester hydrolases, "leucine" aminopeptidase, and succinate, lactate, and glucose-6-phosphate dehydrogenases. These dehydrogenases are key enzymes in the metabolism of carbohydrates in vertebrates. The ultimate goal of these investigations is increased knowledge of the physiology of economically-important coccidian parasites of livestock that will enable researchers to develop more effective drugs for their control.

New antigen to detect Trichinella spiralis in living hogs. A new antigen for use in skin testing swine for trichinosis is being developed at the Beltsville Parasitological Laboratory. Trichina cysts were freed of muscle tissue and fragmented by sonication. This material was then suspended in buffered saline and injected intradermally into normal and infected hogs. Trichinous hogs were tested 7, 15, 21, 28, and 36 days after ingesting 500 encysted trichinae per pound of body weight. Comparable nontrichinous control hogs were injected at the same time. Positive reactions were observed in all trichinous hogs tested from 20

minutes to 24 hours after injection. The nontrichinous hogs had negative results. Injections of the buffered solution alone produced negative reactions in both groups of hogs.

Plans for cooperative trichinosis pilot project being developed. The George A. Hormel Company has provided a room in its Fort Dodge, Iowa, plant for the pilot project and will furnish utilities for one year's operation. Most of the equipment and many supplies have been ordered and some have been delivered. A veterinarian has been found to head the project at Fort Dodge. Livestock Conservation, Incorporated, the cooperator on the project with ARS, is concluding agreements with Wilson Laboratories and Cudahy Laboratories which will jointly provide pepsin for the project. July 1, 1968, has been set as the tentative starting date.

A new anthelmintic, l-tetramisole, proved highly effective against infections of the nodular worm, Oesophagostomum dentatum, in miniature swine. Preliminary results indicated that, at feasible dose rates, l-tetramisole was almost totally effective in removing the nodular worm burdens from experimentally-infected swine.

Natural infection of lambs on pasture with thread-necked strongyles, Nematodirus spp. protects them against subsequent exposure to these parasites as yearlings and older ewes. Fecal samples collected in the fall from lambs at the Meat Animal Research Center at Clay Center, Nebraska, contained almost as many Nematodirus eggs as eggs of the large stomach worm, Haemonchus contortus. These infections were acquired by the lambs during the first grazing season. However, relatively few Nematodirus eggs and large numbers of H. contortus eggs were observed in the feces of sheep collected during the second and subsequent grazing seasons. This finding indicated that the initial exposure and infection with Nematodirus spp. protected the sheep in question against reinfection with these parasites, whereas, similar exposures to infection with H. contortus failed to develop resistance against this nematode.

Two new anthelmintics were tested for the first time against common helminth parasites of turkeys. One, l-tetramisole, proved highly effective against the large roundworm, Ascaridia dissimilis, the cecal worm, Heterakis gallinarum, and the intestinal threadworm, Capillaria obsignata. This is the first anthelmintic that has been effective against the intestinal threadworm of turkeys. In concomitant trials, parbendazole was equally effective against H. gallinarum but comparatively ineffective against A. dissimilis and C. obsignata.

In equine piroplasmosis the carrier state of Babesia equi persisted longer than that of Babesia caballi. Of the two species of Babesia that cause

piroplasmosis in horses, Babesia equi persisted in animals much longer after recovery from the acute stage of the disease than did Babesia caballi. In parasitized horses held in isolation in the Beltsville Parasitological Laboratory, those that recovered from Babesia caballi infections lost the "carrier state" one to three years after infection, whereas those that recovered from Babesia equi infections have been held three years without loss of the "carrier state." This information on duration of the carrier state for the two infections may be of importance in control work.

Complement fixation test accepted by USDA Animal Health Division and the State of Florida as the official diagnostic test for equine piroplasmosis. This test, developed and standardized at the Beltsville Parasitological Laboratory, has been accepted for use in the piroplasmosis control program in Florida and as the official test for this disease in equine animals intended for international and interstate shipment.

The names of parasites of man and animals in Vietnam, Thailand, Laos, and Cambodia have been compiled from published reports, and a host list and bibliography prepared for publication. In order to supply medical, veterinary, and research personnel with a working tool of what is known and what is unknown about the parasites of Vietnam and neighboring countries, the Index-Catalogue of Medical and Veterinary Zoology was searched for information on parasites reported from these countries. A 72-page bibliography and a 38-page list of the parasites arranged by host were prepared for publication. This task was undertaken to assist the war effort, and to help the economic development of these countries.

Utilization of triple-labeled radioisotopes to determine residue(s) of a pesticide and its metabolites in the tissues and fluids of exposed animals and poultry. This capability may reduce the time required up to 90 percent to that needed in the past by research chemists. Initial studies involve a member of the dithiocarbamate fungicide compound group in which the carbon, sulfur, and hydrogen elements are labeled.

CONTROL OF DISEASES OF LIVESTOCK AND POULTRY
(RPA 211)

USDA and Cooperative Program

Location of Intramural Work	Commodity	Scientist Man-years FY 1968
Iowa (Ames)	Cattle	37.2
Maryland (Hyattsville)	Cattle	1.0
Colorado (Denver)	Cattle	1.0
New York (Greenport)	Cattle	27.0
Kenya (Muguga)	Cattle	2.0
Iowa (Ames)	Sheep and goats	3.1
Colorado (Denver)	Sheep and goats	3.0
New York (Greenport)	Sheep and goats	0.6
Michigan (East Lansing)	Poultry	0.3
Iowa (Ames)	Poultry	10.9
Georgia (Athens)	Poultry	5.0
Mississippi (State College)	Poultry	1.0
New York (Greenport)	Poultry	1.3
Iowa (Ames)	Swine	13.6
New York (Greenport)	Swine	4.4
Kenya (Muguga)	Swine	2.0
Iowa (Ames)	Horses	1.3
New York (Greenport)	Horses	0.7
Washington (Pullman)	Fur-bearing animals	3.0
	Total	118.4

Intramural program is supplemented by extramural support representing (a) 13.1 SMY's at State Agricultural Experiment Stations and Universities and (b) P.L. 480 funds in six countries.

Problems and Objectives

Infectious diseases represent the single greatest hazard to the production of an adequate and wholesome supply of animal protein. This hazard increases as the prevalence and severity of a disease increases. The total losses from animal diseases will exceed $2.6 billion annually by 1980 if continued at the present rates. Losses result from mortality, reduced productivity, cost of treatment or immunizations, cost of regulatory programs, and condemnations at the slaughterhouse. Some animal diseases are also transmissible to man.

Research objectives include studies of:

1. The nature of infectious agents.
2. Mechanisms of disease resistance and immunity.
3. The role of environment, genetics, and infectious agents in the etiology of diseases.
4. Methods of diagnosis, prevention, treatment, and control of infectious diseases.
5. Methods of keeping foreign diseases out of this country.

Progress - USDA and Cooperative Programs

A. Cattle

1. Vibriosis. Bacteriophages were isolated from 18 of 38 strains of Vibrio fetus. One isolate was characterized as to physical tolerance, growth cycle, morphology, and host range.

Endotoxin was extracted from V. fetus cells with 45 percent phenol and chemically characterized. It consisted of lipopolysaccharide that could be separated into lipid A and seven free fatty acids. In mice, the lipopolysaccharide was considerably more toxic than was lipid A.

2. Tuberculosis. Experimental Mycobacterium bovis infections were studied in cattle following vaccination with M. paratuberculosis bacterin.

In cooperation with the College of Veterinary Medicine at Michigan State University, a study was conducted on the effect of an intradermal tuberculin test on monocyte migration-inhibition tests. Results suggest that the skin test did effect the migration of cells from noninfected skin-tested animals. Results also suggest that supernatant fluids from sensitized lymphocytes cause inhibition of normal macrophages or normal peritoneal exudate cells in the presence of PPD whereas supernatant fluids from normal lymphocytes fail to produce the same results.

Under a P.L. 480 research grant to scientists at the Vallabhbhai Patel Chest Institute, Delhi, India, the nature and composition of M. tuberculosis is being studied.

3. Paratuberculosis. The intradermic johnin test, the complement fixation test, the gel diffusion test, microscopic examination of fecal specimens, and culturing of fecal specimens for M. paratuberculosis were evaluated as methods for detecting paratuberculous cattle.

It was found that the serological, allergic, or microscopical examinations used individually or together were of little value in detecting preclinical paratuberculous cattle. Cultural examination of fecal specimens was the most accurate method of detecting paratuberculous cattle.

The incidence of Johne's disease and the ages at which clinical signs were observed were compared for several years in two herds, one kept under relatively poor sanitary conditions (herd A) and one under good sanitary conditions along with a calf isolation program (herd B). The annual incidence of clinical Johne's disease was 4.2 percent in herd A and 1.4 percent in herd B. The highest incidence of clinical Johne's disease was observed in 6- and 7-year old cattle in herd A and 6-, 8-, and 9-year old cattle in herd B. Good management, good sanitary practices, and raising calves separately from the adults greatly reduced losses from the disease in herd B, but did not eliminate the disease. It was also noted that cattle with clinical signs of Johne's disease in both herds were older than those reported by others.

4. Enteric diseases. Multiple freeze-thaw or sonication of cytopathogenic (CP) strains of bovine viral diarrhea (BVD) virus did not change its filtration properties, but did increase filterability of noncytopathogenic (NCP) strains. Frozen storage (-75°C.) from 1 to 4 years, which included a single freeze-thaw, increased the filterability of CP, but not of NCP strains.

Filtration of viral concentrates through agarose beads and chips indicated the presence of infectious particles of about 30-40 and 80-100 mμ sizes. These data confirmed earlier filtration and preliminary electron microscopic studies.

Electron microscopic studies on BVD virus and its soluble antigen (SA) revealed particles of 80-100 and 15-20 mμ, respectively.

Neonatal calves experimentally infected with BVD virus were immune to rechallenge inoculations and did not have immunological tolerance, with the exception of two calves that failed to produce viral-neutralizing antibodies for 42 and 73 days.

Preliminary tests on a noninfectious experimental vaccine for BVD indicated high levels of neutralizing antibodies in serums of vaccinated cattle. Comparative tests of experimental vaccines are continuing.

5. Respiratory diseases. Morphological, cultural, biochemical, and serological characteristics of 43 strains of Pasteurella hemolytica, isolated from the respiratory tract of cattle (including 37 that had shipping fever) were determined. With one exception, all strains satisfied the major criteria for differentiation of the species from Past. multocida--hemolysis on blood agar, absence of indole production, and growth on MacConkey's agar. Many strain variations in fermentative capacity were noted, although other biochemical characteristics were relatively constant. The ability of a strain to ferment carbohydrates often depended upon the medium in which the test was conducted. All strains studied were identified as type A strains on the basis of colonial morphology, biochemical and growth characteristics, and sensitivity to penicillin. When grouped on the basis of serological reactions, 38 strains were type 1, three were type 2, one was type 11, and one was untypable; 34 of the shipping fever strains were type 1 and three were type 2.

Aerosol exposure to Past. hemolytica, suspended in a broth containing high molecular weight dextran sulfates, did not produce pneumonia in calves. It was demonstrated previously that the dextran sulfates increased the susceptibility of mice to Past. hemolytica infection.

Two plaque types of the SF-4 strain of parainfluenza-3 virus are being characterized. An experiment is underway to determine whether each will cause a clinical response in calves and cross-protect against the other type.

A virus neutralizing substance that appears in nasal mucus of calves after aerosol exposure to parainfluenza-3 virus is being characterized, and its role in immunity studied.

The specificity of the immunity produced in mice to live challenge inoculation and the specificity of the protection to the endotoxins from three strains of Past. multocida by injection of these endotoxins were studied. Endotoxin tolerance was obtained after daily injections of the Past. multocida endotoxin, but the "tolerant" mice were not immune to live challenge inoculation. Specific immunity to live challenge inoculation, and specific protection to challenge inoculation with endotoxin was obtained after four intraperitoneal injections at 10-day intervals. If the endotoxins were first injected intraperitoneally, and the challenge inoculation given intravenously 10 days later, the mice developed anaphylactic shock. About 20 percent died within an hour, but the others

were nonspecifically protected to the toxins. However, a single injection of 10 µg. of the endotoxin from one strain protected 100 percent of the mice to an intravenous (i.v.) challenge with the same toxin given 10 days later that killed 100 percent of the controls. Equally good protection was obtained to live challenge inoculation. Considerably poorer protection to challenge with toxin by the i.v. route was obtained with all the other toxins.

Adsorption of antigens from the gut as shown by serological test and allergic reactions is well documented, but little has been reported on the stimulation of active immunity by oral administration of killed micro-organisms. Experiments were designed to determine the immunological and serological responses in chickens and turkeys and the immunological response in mice to killed Past. multocida when administered perorally. Active immunity was induced in chickens and turkeys but not in mice by oral administration of killed Past. multocida. The amount of vaccine required to induce immunity by this method was relatively large compared with parenteral administration, and these doses were more effective than one or two doses. Agar-double diffusion and serum-plate-agglutination tests did not show any correlation with the active immunity obtained against the live organism. Prechallenge serums from most birds that were immune were serologically negative.

Studies under a P.L. 480 research grant at the Institute for Pathology and Therapeutics at Belgrade, Yugoslavia, have resulted in the isolation of four different viral agents from cases of respiratory disease in cattle. Two of these have been characterized and identified as infectious bovine rhinotracheitis (IBR) virus and myxovirus parainfluenza-3 (PI-3). The disease produced by artificial infection with IBR virus was milder than the naturally-occurring disease.

6. Mastitis. Factors that might affect the growth of Streptococcus agalactiae in milk were studied. Highest inhibitory titers were obtained when the diluent milk was steamed at 100°C. for 30 min. The inhibitory titer of pasteurized milk diluted in steamed milk was progressively increased when increasing quantities of ammonium thiocyanate per ml. was added to both milks. A stimulatory substance that counteracted the inhibitory activity of milk could be removed from whey by dialysis or ultrafiltration. Colostrum samples tested had more stimulatory but less inhibitory substance than milk. A radiographic technique was developed for in vivo determination of the anatomy of the bovine teat by injecting through the teat canal a suspension of barium sulfate. Canals lengthened and dilated with increase in age. Vacuum fluctuation at the teat end during mechanical milking was studied and found to be caused by type of pulsation, amount and location of air admission, and internal diameter of the milk collection system.

Cooperative studies on mastitis at the School of Veterinary Medicine, University of California, revealed that endotoxin extracted from Aerobacter aerogenes would produce all the local and systemic signs as were produced by viable or heat-killed organisms. However, the clinical signs of mastitis appeared much more rapidly following intramammary injection of endotoxin. Also, the initial changes in the milk were more drastic with endotoxin than with viable organisms. It was also learned that serum of calves from normal cows and even bovine fetuses from the third trimester, was bactericidal against A. aerogenes, Escherichia coli, and Pseudomonas aeruginosa. The presence of bactericidal serum factors in milk appears to be strictly a function of the bacterial strain involved and of milk from individual quarters.

A survey of several phage isolates from various strains of Staphylococci disclosed that there were at least two geometrically distinct head types (simple polygonal and oblong forms). The tails were noncontractile and bore well-defined baseplate assemblies with pentagonal symmetry. The tail length was highly variable (0.15->1 μ) and was independent of the geometry of the phage's head.

A unique complex structure consisting of a double membraned central body (50-80 mμ) bearing numerous uniform fibrillar projections (90-110 mμ) was fortuitously encountered in a single crude lysate of T-4 infected Escherichia coli B.

A study to determine the pathogenesis, immunity, and treatment of ovine staphylococcal mastitis has begun at the Veterinary Institute, Beit Dagan, Israel, under a P.L. 480 grant.

7. Epizootic bovine abortion. Crude chlortetracycline (CTC), in a dosage of 2.5 g./day/cow given prophylactically as a feed additive beginning just before the time of infection (approximately midgestation) and continued until the termination of pregnancy, prevented abortion in cattle from the epizootic bovine abortion (EBA) agent. Serological evidence indicates that the infection does not become established in cattle on this dosage of the antibiotic. In a current trial in which a daily dosage of 1 g. of the crude antibiotic was employed, from 25 to 35 percent of the cattle have thus far aborted. It was found that the addition of sulfamethazine to the feed additive does not potentiate the therapeutic effectiveness of CTC. These results provide a basis for further studies which promise to lead to a practical form of prophylactic therapy for the control of EBA.

8. Foot rot. A cooperative project was initiated at the University of Missouri at Columbia to intensively investigate cases of foot rot of cattle. This study was designed to: 1) determine the epidemiologic factors

involved in foot rot, 2) definitely characterize the clinical disease, 3) determine the bacterial flora associated with foot lameness of cattle, and 4) consistently transmit foot rot to susceptible animals.

Epidemiological forms were designed, suitable methods were selected for obtaining tissue samples, and satisfactory techniques were developed for isolating the bacteria. Thirty-nine cases of naturally-occurring foot lameness were investigated during the first year of this study. Of these, 25 were clinically diagnosed as typical foot rot. Animal owners were interviewed, their premises observed, and the epidemiological information obtained was recorded on a prepared form. Each animal was cast, carefully examined, and a tissue sample was obtained for microscopic examination and bacterial culture. A blood sample was collected for a complete blood count and later serological study.

Four groups of four calves each were used in an initial experimental transmission study. A specific bacteria, Spheropherus necrophorus, was used to infect one group. A second group was infected with material from a natural case of foot rot. A third group was infected with bacteria grown in artificial mediums from a natural case of foot rot. The fourth group was infected with infectious material from a bovine liver abscess. Preliminary evidence indicates that infection was transmitted to most animals in this initial experiment transmission study.

9. Leptospirosis. Adequate immunity, including protection against renal infection, required the use of motile, metabolizing leptospires that were nonreplicating (because of exposure to ionizing radiation or streptomycin), and living cultures of avirulent strains.

Such cultures were safe (did not initiate renal leptospirosis or cause nephritis) and protected hamsters, swine, and cattle against clinical leptospirosis including abortion and renal leptospirosis. One ml. of a 1:160 dilution of vaccine adequately immunized hamsters for 3 months and 5 ml. protected swine for 7 months, the longest time tested.

Rifamycin and doxycycline did not cure hamsters of renal leptospirosis; and oral or subcutaneous doses of doxycycline did not cure swine. However, a single dose of dihydrostreptomycin (25 mg./kg.) cured both swine and cattle.

Glucose stimulates the growth of pathogenic leptospires. The substrate concentration of D-glucose has been related to respiratory rate (Q_{O_2}) of Leptospira pomona strain DM_2 cells. Considerable structural specificity was observed in the ability of L. pomona cells to oxidize various sugars and sugar derivatives. Supplementation studies indicated that ATP or Mg^{++} did

not significantly affect the oxidation of glucose by resting cells. Hexokinase activity highly specific for D-glucose was demonstrated in cell-free extracts of L. pomona and was enhanced by additions of ATP, TPN, and Mg++.

A leptospiral strain was found that would initiate growth in vitro at temperatures of 37°C. or above.

Lipids were extracted from virulent and avirulent L. pomona and purified. The haptenic material was neither dermalnecrotic nor lethal but it inhibited the growth of normal mice, killed peritoneal macrophages, and hastened the death of hamsters when simultaneously exposed to virulent L. pomona.

A soluble, thermolabile, nondialyzable, toxic factor was demonstrated in living and disrupted L. pomona and in the supernatant fluids of cultures by effects on peritoneal macrophages. It was not neutralized by specific antiserum.

10. Lymphosarcoma. A viral agent inducing syncytia formation in monolayer cultures of bovine embryonic spleen cells and a rabbit cornea cell line was isolated from cattle with lymphosarcoma. The agent was strongly cell-associated and transmissible with cell-free filtrates at very low dilutions. An antigen prepared from heavily infected cultures produced precipitin lines by immunodiffusion when tested against serums from lymphosarcomatous and apparently normal cattle. Buffy coat cells and cellular elements present in milk from apparently normal cattle whose serums gave a positive precipitin reaction contained the virus. Some non-reacting offspring of dams that reacted were also virus carriers. Fluorescent conjugates prepared with precipitating serums stained infected cells specifically while nonprecipitating serums did not. Electron microscopic examination of ultra-thin sections from infected cell cultures revealed virus-like particles budding at the cell membranes in a manner resembling the avian and murine leukemia viruses. Morphologically, these virus-like particles bore some resemblance to the mouse mammary tumor virus. Evidence that the virus plays a role, if any, in inducing bovine lymphosarcoma has not been established.

Under a research contract at the University of Nebraska, a case of leukemia involving primarily the skin was studied. Histopathological examination of the papilloma-type lesion revealed that the tissue consisted primarily of lymphocytes. Animal inoculation studies using skin grafts and macerated tissue are in progress. Studies are also in progress on animals inoculated with material from other field cases.

An additional investigation was made of animals on a farm in northern Nebraska where a human case of leukemia was reported. No history was available that leukemia cases had occurred in the animals during the last 3 to 5 years.

In cooperation with the Animal Health Division, the epizootiology of bovine lymphosarcoma is being studied at the University of Pennsylvania.

Under a research contract at the New York State Veterinary College, partially purified and concentrated tissues from cattle with bovine leukemia have been injected into rabbits. The antiserums produced in rabbits are now being studied against various antigens by using the agar gel diffusion and fluorescent antibody methods.

The cause and development of bovine lymphosarcoma are being studied at the Indian Veterinary Research Institute, Izatnagar, India, under a P.L. 480 research grant.

11. Bloat. Radiotelemetric equipment has been designed, built, and tested to continuously transmit the pressure changes in any selected location of the digestive tract. This permits monitoring of intraruminal pressures during all stages of severe and fatal bloat without restraining the animal.

During grain engorgement, intraruminal gas production is minimal. Bloating is not a complicating factor in uncomplicated grain engorgement. We have also recently found, in a pilot experiment, that soaking the grain (corn) before putting it in the rumen enhances the symptoms of grain engorgement.

Micro-methods of blood gas analysis are now being used to increase our knowledge of blood gas changes in ruminants since blood sampling can be done more frequently.

Work has been done recently on the diffusing capacity of the lungs of sheep in different states of health. The carbon monoxide method of testing for diffusing capacity in general is not applicable to ruminants because of their normal irregular breathing patterns and because of the pulmonary absorption of eructated gases during eructation.

A method was developed to examine the "fit" of the ruminal microbial population to the diet of the animal. Such a method is needed for studies on the rate of microbial adaptation to high grain rations and factors affecting this adaptation. In vitro gas production rates with hay or grain as substrate were measured with ruminal contents from sheep fed either hay or grain. Fermentation rates were different and characteristic for each ration. One day after rations were changed from hay to grain and vice versa, rates characteristic of the new ration were measured. It is unlikely that the microbial population had fully adapted to the new diet so rapidly. It seems rather that the method needs to be modified if it is to be used for the purpose intended.

Several genera of important ruminal bacteria carboxylate certain acids to synthesize amino acid carbon skeletons. The biosynthetic pathways are different from any previously described in other organisms and the biochemical mechanisms are not known. We have studied the carboxylation of

isobutyrate to synthesize the carbon skeleton of valine using cell-free extracts of Peptostreptococcus elsdenii. Evidence has been obtained that ferredoxin or flavodoxin is required as low potential electron carrier.

Evidence was also obtained for involvement of coenzyme A, adenosine triphosphate, and thiamin pyrophosphate. A sequence of reactions for the reductive carboxylation, based upon studies of valine biosynthesis from isobutyrate, has been proposed. Extracts from another ruminal bacterium, Bacteroides ruminicola, also carboxylated isobutyrate. Ferredoxin could not be detected in extracts from this organism, and pyruvate, but not hydrogen, was required as source of low potential electrons.

A technique for hypophysectomizing calves has been developed which will permit the biochemical and anatomic changes that occur after hypophysectomy and the secretory function of the adrenal cortex to be characterized.

A method for estimating concentrations of adrenal corticosteroids in peripheral blood of cattle has been adapted and characterized. When this technique was applied to a study of parturient paresis in dairy cows, increased concentrations of adrenal corticosteroids were found in this condition. A repression of insulin secretion has also been demonstrated.

12. Brucellosis. A comparison of agglutinins for Brucella separated from serums and milk wheys of infected animals by density gradient ultracentrifugation indicates an appreciably more complex mixture of agglutinins in whey as compared to the agglutinins in serum. In addition, the chemical and serological properties of some of the agglutinins in whey differ significantly from those commonly present in serums. This research provides background information for studies on the nature of agglutinins in milk from cows that are responsible for false positive reactions commonly encountered with the ring test on herd milk samples under field conditions.

The results of a cooperative study at the University of Wisconsin indicate that the majority of the antigenic specificities of early serum immunoglobulins are directed against the external Brucella lipopolysaccharide antigens. Upon repeated exposure to the bacterial cell, the immunoglobulins develop antigenic specificities against the internal as well as the external antigens of the Brucella cell.

13. Pinkeye. Irradiation of cattle eyes with a mercury sunlamp enhanced the effect of Moraxella bovis infection. The optimal results were obtained by irradiating the eyes twice for 10 minutes, 24 hours apart, from a distance of 61 cm. After the second irradiation, instillation of M. bovis resulted in infection and corneal opacity. Eyes of cattle were reexposed to homologous and heterologous strains of M. bovis. By comparison with the results of initial exposure, the reduction in the infection rate and in the number of eyes developing keratitis was considered

a reflection of enhanced resistance. This, in turn, indicated that bovine infectious keratoconjunctivitis may be amenable to prophylactic measures. Nonhemolytic M. bovis did not cause keratitis but could spontaneously revert to the hemolytic form which did cause keratitis. Prolonged infection with nonhemolytic M. bovis did not produce resistance to infection with the hemolytic form.

14. Bluetongue (BT). Negative-staining electron microscopy of bluetongue virus (BTV) confirmed its underlying reovirus-like symmetry composed of large pentagonal morphological units. In addition, this work disclosed that mature BTV virions are very complex particles. They consist of a reovirus-like core (55 mμ) surrounded by a 12 mμ shell of fibrillar components firmly attached to the core. These fibrils terminate at a membranous envelope which may also bear 10 to 12 mμ uniform surface projections. Fluorocarbon treatment appears to disrupt the membranous envelope. The minimum number of virion-specific antigens of the mature BTV is 5.

Bluetongue virus produces a specific type of lesion in cultured cells, including the development of two types of intracytoplasmic inclusion bodies. The virus replicated in association with the second type of inclusion body. These bodies (viroplasms) were composed of ribonucleic acid and had a high content of bluetongue viral antigen, as they reacted positively to BTV specifically-tagged viral antibodies. Mature virus was never present within the nucleus.

The viroplasms (type II inclusion bodies) formed in the cytoplasm in response to BTV infection were observed in both infected primary lamb kidney cells and the salivary glands of Culicoides variipennis (the vector of BT). Macrotubules are observed in association with these viroplasms. These tubules are believed to be bluetongue viral protein, and evidence indicates that these tubular protein sheaths are manufactured by altered cellular endoplasmic reticula.

These discoveries demonstrate that BTV replicates similarly in both cultured cells and insect vector tissues.

Nine successful serial transmissions have occurred in sheep via bites of C. variipennis and by artificial inoculation from October 1967 to June 1968. The infection rate of the Culicoides varied from 2.5 percent at the first passage level to 65.1 percent at the seventh passage level. During the winter months of December through March, the infection rate varied from 26 percent to 40 percent. The virulence of the BTV appeared enhanced at the 1st, 2nd, 4th, and 7th passage levels. The same initial virus, but passaged serially by artificial inoculation on a comparable basis, has produced a constant moderate BT clinical response.

Eight species of small mammals and 16 species of biting insects were obtained in the immediate vicinity of two bluetongue epizootics and tested for the

presence of BTV. All of the mammals and insects were negative for isolation of BTV in embryonating chicken eggs and/or BT-susceptible sheep. In both epizootic situations, a portion of the flock of sentinel sheep acquired immunity against BTV challenge.

Bluetongue epizootics were investigated and the pathogenesis of natural BT infection in cattle was described.

The research studies are important in evaluating the role of C. variipennis in epizootics of BT and their disease transmission potential. Also, the data indicate problems associated with the biological and reservoir characteristics of the virus and its detection.

15. Foreign animal diseases. Cattle vaccinated with acetylethyleneimine (AEI) inactivated and purified 140 S A-1, O-2, and C-3 viruses in monovalent and trivalent forms resolved some basic immunological questions. A trivalent vaccine was prepared by mixing equal amounts of each of these three purified antigens. Each virus in the trivalent form acted independent of the effects of the other two viruses. There was a significant difference in antibody response and resistance to infection among different types of foot-and-mouth disease virus (FMDV).

The effect of several factors on the susceptibility of cultured cells to FMDV has been investigated. Cultures prepared from kidney cells from two different animals of the same species varied as much as twofold in plaque production with FMDV. There was no evidence that mixing of kidney cells from two different animals of the same species resulted in incompatibility for cell growth and susceptibility to FMDV. Primary bovine kidney cells grown in serums from different steers possessed different susceptibility to FMDV, especially low tissue culture passage virus. Cell dispersion with trypsin by conventional methods was investigated in detail in developing methods for more susceptible cultures.

There were wide differences in stability of FMDV in serums from different normal steers indicating the importance of pretesting of serums for nonspecific neutralizing activity as well as effect on cell susceptibility.

Methods were developed for preparing trypsin-dispersed bovine kidney cells and swine kidney cells that retain a high degree of viability during freezing, storage, and thawing in large quantities at high concentrations.

Information was obtained on the mechanism of the infection of baby hamster kidney-21 (BHK) cells by FMDV.

Inhibition of protein turnover in infected cells was lowest at 25°C. and greatest at 41°C. Protein turnover in infected cells was always maximal at

33°C. Virus production was about equal in cells incubated at 30°C. for 12 hours, at 37°C. for 5 hours or at 41°C. for 4 hours. Viral-specific ribonucleic acid (RNA) polymerase in infected cells and its cell-free activity were both maximal at 37°C. However, extended incubation at lower temperatures of the cell-free system also raised the concentration of the viral RNA products to maximal levels. Sucrose gradient profiles of the products of RNA polymerase prepared and assayed at different temperatures showed no qualitative differences.

The site of FMDV-RNA synthesis was in the cytoplasm where both the viral RNA-enzyme complex and the viral RNA products derived from the complex were fixed to membranes. The RNA-enzyme complex was freed by detergent from the membranes and was split into two active components, one synthesizing the 20 S double-stranded (ds) RNA found only in cells and one synthesizing the single-stranded (ss) 37 S RNA of the viral core. Both of the RNA forms were infectious, but the ratio of infectivity to mass was several orders of magnitude lower for ds than for ss RNA.

Infection of either BHK cells or primary calf kidney monolayers by FMDV inhibited the methylation of both transfer RNA and the nuclear RNA, the precursor of ribosomal RNA. This inhibition occurred by an alteration in the activity of cellular RNA methylase, since the molecular precursors for methylation were not affected. Such submethylation markedly diminishes the cells' capacity for synthesizing their normal complement of proteins but permits the synthesis of viral-specific proteins.

Polyribosomes (the subcellular units required for protein biosynthesis) have been isolated from FMDV-infected and noninfected cells and characterized with regard to their molecular sizes.

Three different exotic viruses of livestock have been characterized by electron microscopy (EM) during their morphogenesis in tissue culture cells.

Intracellular crystalline forms comprised of either complete FMDV particles, type A_1, or of particles lacking their internal ribonucleic acid cores, were seen by EM to combine only with antibody to type A_1 virus, and not to normal gamma globulin or to antibody against virus of other immunological types. The combination of the virus with its specific antibody was visualized by means of electron-opaque ferritin molecules which were chemically bound to antibody. This study confirmed the intracellular existence of crystalline arrays of both FMDV and of coreless viral shells which possess antigenicity similar to that of whole virus particles.

When hydroxyurea was present during the multiplication of African swine fever virus in tissue culture, virus particles without deoxyribonucleic acid cores were the predominating morphogenic units. Their formation is concomitant with as much as a 10,000-fold drop in infectivity. There was no change, however, in the amount of complement fixing antigen which was synthesized.

Electron micrographs of tissue culture cells infected with African horse-sickness virus show virus particles with the characteristics of the arbovirus group.

Glutaraldehyde offers much as a surface disinfectant or viricide against FMDV at low concentrations and at lower as well as at higher temperatures. In concentrations of 0.1 to 1 percent of the chemical, from 6 to 7 logs of viral activity was inactivated at 37° to 4°C. in 1 hr. as assayed in tissue culture. However, inoculation of mice with similar material indicated the presence of active virus in some preparations. This difference was attributed to the cell regulator property that keto-aldehydes are said to possess, which would give a false test for inactivation.

Silicone-impregnated butyl rubber stoppers for vaccine bottles are much superior to plain rubber stoppers for retaining vacuum and dryness of the viral preparations, freeze-dried and stored, as determined by persistence of infectivity of FMDV and vesicular stomatitis virus preserved in this fashion. Random drying of antiserum on paper and freeze-drying samples of the same antiserum in bottles, followed by similar conditions of storage, indicated the methods to be about equal for preserving antibody titer, temperature for temperature. Freezing, thawing, freeze-drying, and storage were not conducive to preserving purified virus of FMDV in the range of -80° to 37°C. as compared to similar treatment of crude virus.

Foot-and-mouth disease virus has been further characterized with respect to its chemical and physical properties. Purified FMDV has been produced in quantities of 0.1 g./week. In addition, 115 BHK cell cultures (ca. 800 million cells per culture) were produced each week for use in immunological and viral replication studies. Medium preparation vessels were received which should permit the future production of up to 0.5 g. of pure virus per week.

A large plaque variant of a standard high-passage tissue culture FMDV parent, type A-119, shifted in density from 1.43 to 1.49 g./ml. on heating at 55°C. for 15 min. in 3.5 M cesium chloride, whereas only a portion of the population in the parent virus did so. Virus particles also increased in buoyant density when frozen in 3.5 M cesium chloride. These intermediates with increased density that form during the degradation of FMDV are presumably caused by temperature-induced changes in the RNA configuration of the virus core. When heated at 60°C. for 15 min., the large plaque variant was degraded while two zones of the parent virus remained, indicating both heterogeneity and partial resistance to heating.

The sedimentation rate (s-rate) of RNA extracted from density-shifted viruses was 20 percent higher than from unshifted virus. Extracted RNA from the parent virus exhibited no increase in s-rate on heating to 60°C. in

absence of added salt. The Programma 101 desk-top computer was used to process the analytical ultracentrifuge optical records. In addition, several computer programs for analyses of moving boundary and equilibrium centrifugation methods were written.

Information has been obtained on the homogeneity, size, and N-terminal amino acid composition of FMDV protein. These studies are directed toward determining the sequence of the amino acids in FMDV protein, especially of its immunogenic groupings. The phenol-extracted pure virus protein was solubilized by malylation and subjected to gel filtration on Sephadex G150. By calibrating the column with malyl derivatives of several proteins of known molecular weight, notably trypsin and pepsin, the malyl FMDV peptide was estimated to be 31,500 daltons. However, gel electrophoresis of the FMDV malyl peptide gave two zones that were partially resolved by gel filtration.

Dansylation of either the peptide or malyl peptide gave no detectable N-terminal dansyl derivative. As further evidence of the peptide having no free N-terminal α-amino groups, digestion of peptide or intact virus with leucine aminopeptidase (LAP) released no detectable amino acid residues. Enzymatic digestion of malyl protein with LAP, however, gave trace amounts of valine, glutamic, and aspartic amino acids. This may have been due to partial hydrolysis of an N-terminal α-amino acyl linkage during the malylation.

Three antigenic variants of FMDV, type A, strain 119 (FMDV, A-119) were isolated, purified, and characterized. One of the variants contains what are termed a- and b-determinant sites (140 S-ab). Virus of this antigenic structure appears to be the wild or animal form of FMDV. Another virus variant contains the b-determinant site (140 S-b) while the third variant contains a b-like determinant site (140 S-Sm.pl.). Antibody reacting with the a-determinant does not neutralize the virus. Thus, FMDV apparently contains both critical and noncritical neutralizing sites. These studies demonstrate that FMDV contains a mosaic of different antigenic groupings on its surface, the amount and distribution of which may influence its pathogenicity and its potential efficacy for vaccine purposes.

The three antigenic variants could only be demonstrated with early infection serum which contains 19 S antibody. Late infection serum containing 7 S antibody could not differentiate these variants. Studies indicate that the differing reactivity of 19 S and 7 S antibodies is due to the fact that the 19 S antibodies have a smaller combining site than 7 S antibodies. These observations could contribute significantly to our antigenic analyses and subtypes of FMDV, to problems of cross-reactivity, and basic information on the mechanism of antibody formation.

Steers singly infected with type A, FMDV and steers doubly infected with type A followed later by type O virus, which had become carriers, were investigated for biological and antigenic changes in the virus. Virus isolates from such animals obtained from zero to 160 days postinfection were examined by a number of tests. The results of such tests were compared with results of similar tests performed on the parent infecting virus. Only strains from the same animal were compared. Differences in both biological and antigenic nature were found which were attributed to the sojourn of the virus in the animal body.

A process of adsorption with homogenized mouse kidney and propagation of the unadsorbed virus in bovine kidney cell cultures was used to obtain FMDV that was relatively avirulent for adult mice. When inoculated in adult mice, this virus stimulated the formation of antibody that neutralized the original virus. The adsorption technique is now being used in attempts to obtain virus that will stimulate formation of antibody against FMDV in cattle without producing the disease.

Comparisons of adsorption by mouse brain, lung, and kidney demonstrated that the active component in brain was different from that in kidney and lung. There was less adsorption activity per gram of brain, and the brain component was sedimented by centrifugation and inactivated by heat more easily than the component in kidney and lung. In addition, virus did not attach as securely to brain and the attached virus was recovered in larger amounts than from kidney.

Adsorption activity of mouse muscle varied greatly between experiments in relation to the temperature at which the tissue was held before being processed. If muscle was minced and kept in an ice bath for 30 min. before processing, it adsorbed less virus than muscle incubated for the same time at 37°C. Possibly,this was due to enzymatic effect on virus receptor sites.

Two plaque type variants of foot-and-mouth disease virus (FMDV, A-119) have been isolated from virus stocks passaged approximately 120 times in bovine kidney cell cultures and once in baby hamster cells. The small plaque (SP) virus forms plaques at 72 hr. that are $\leq$ 2 mm. in diameter, while the large plaque (LP) virus forms plaques that average 4-6 mm. in diameter. Plaque formation by the SP virus is inhibited under normal agar overlay medium, but the addition of diethylaminoethyl (DEAE)-dextran to agar medium removes this inhibition and large plaques are formed. The LP virus is relatively uninhibited under normal agar medium. Reciprocal cross-neutralization tests with 28-day postinoculation anti-LP and anti-SP guinea pig serums and LP and SP viruses gave results indicating that the viruses are distinct antigenically. The LP variant was pathogenic for suckling mice and killed mice in a normal titration pattern within 3 days. In contrast, the titration pattern of the SP virus was erratic and the latent period of death for mice was longer.

These studies demonstrate the intratypic heterogeneity of viruses in some stocks of FMDV and the biological differences that characterize these populations. Practically, these differences can serve as markers to distinguish virulent and less virulent or attenuated strains of FMDV, A-119. Also, it is now possible to utilize the plaque count method to assay the small plaque virus and to detect small plaques that may be present in virus stocks. Basically, these studies may reflect a more intrinsic difference between these viruses that should be considered in any biochemical, biophysical, immunological, or serological study on FMDV, A-119.

Following clinical infection, many cattle become carriers of FMDV. If FMD antiserums or killed virus vaccines are given before exposure to FMDV, the cattle also become carriers often without showing clinical signs or lesions of FMD. Sheep and goats may be made carriers of FMDV by similar techniques. The carrier state in swine has not been demonstrated. In carrier animals, the virus may be found in the throat and nasal areas. Local antibody also occurs in the excretions from these areas and its significance and differences from serum antibody are being studied. The virus may be detected in fluids from these areas by various virus isolation techniques and by fluorescent antibody tests. Although there is circumstantial evidence of spread of FMD by means of carrier animals, laboratory attempts to demonstrate transmission of FMDV from carrier to contact animals thus far have been unsuccessful. Steroid stress, trauma, and roundworm infestation have been used to implement such transmission, but the results to date have been negative.

Studies have recently begun on the genetic and other biological and immunological properties of FMDV under a P.L. 480 research grant at the Hebrew University, Hadassah Medical School in Jerusalem.

Comparative aspects of FMD in Turkey are being studied under a P.L. 480 grant to the Ministry of Agriculture, Ankara, Turkey.

Under a P.L. 480 grant at the Institute of Preventive Veterinary Medicine, Belgrade, Yugoslavia, workers have begun typing the various FMD viral isolates in their repository. They have also started propagating FMDV in several cell culture systems.

Assistance to Animal Health Division was continued in providing diagnoses of diseases suspected of being foreign in origin. The number of specimens received this year was tenfold the total number received in the preceding 4 years. They consisted of tissues and an exceedingly large number of serums from several animal and poultry species. Among the diseases for which they were tested were FMD, vesicular stomatitis, vesicular exanthema of swine, and goat pleuropneumonia. Large numbers and quantities of diagnostic reagents were produced to update and supplement the reagents in the diagnostic repository. In addition, seed virus of duck virus enteritis (DVE) was cloned and propagated and delivered to the duck industry for use as a vaccine. Also, DVE antiserum of high titer was

released to domestic laboratories. Diagnostic capabilities for African horsesickness and DVE were achieved.

Of particular interest is the preparation of guinea pig antiserum for four subtypes of FMD and two new subtypes of vesicular stomatitis virus, exotic to this country, in expanding the number of reagents presently used in the differentiation of vesicular diseases.

The euglobulin antibody fraction of antiserum which was dyed with fluorescein is being used with great success in detecting Mycoplasma mycoides var. mycoides in culture fluids and in lung lesions but not in tissues of infected animals.

Examination of a serum from a steer in the later stages of recovery from contagious bovine pleuropneumonia (CBPP) showed that it contained a complement-fixing (CF) antibody, which was pH 6.0 soluble, found in the upper portion of the sucrose density column and was not reducible by 2-mercaptoethanol (2-Me). This antibody is of the 7 S class. The agglutinating antibody appeared to be divided between the pH 6.0 soluble and insoluble fractions. The agglutinating activity of either of these fractions is not altered by 2-Me and most of the antibody is found in the lower portion of the gradient.

Three DNA preparations from virulent CBPP organisms and two from vaccine strains had a melting point of 74-75°C. Electrophoretic studies and CF tests conducted with pig antiserum did not differentiate between virulent and vaccine strains of CBPP.

16. Mycotic diseases. The antigens present in culture filtrates of Nocardia asteroides have been studied allergenically and serologically. Erythrocyte-sensitizing antigens from N. asteroides reacted with antiserums prepared against Mycobacteria, but hemagglutination inhibition tests demonstrated antigenic differences between Nocardia and Mycobacteria and between species of Nocardia.

The pathology and pathogenesis of mycotic abortion were studied in sheep following intravenous administration of Aspergillus fumigatus. Placental tissues appeared highly susceptible to A. fumigatus infection, while maternal tissues were more resistant to infection. Studies of the pathogenesis of mycotic abortion are continuing following administration of fungal spores by various routes.

B. Sheep and goats

1. Vibriosis. In a cooperative study at Colorado State University, the duration of immunity in ewes vaccinated in 1963 with Vibrio fetus serotype I and V oil adjuvant bivalent bacterin is being determined. The immunity of the ewes is orally challenged during advanced gestation at 2, 3, 4, 5,

and 6 years of age, with the combined V. fetus serotype I and V organisms. Immunity challenge is made each year in nonexposed designated lots of vaccinated and nonvaccinated ewes.

In 1968, the immunity of 52 six-year-old pregnant ewes, divided into three lots, was challenged. In lot 1, one vibrionic abortion resulted from 11 nonvaccinated ewes whose immunity was challenged. In lot 2, the immunity of 21 vaccinated ewes was challenged and one vibrionic abortion resulted. In lot 3, there were no abortions in 20 nonvaccinated, control ewes with nonchallenged immunity.

From data obtained in the preceding 4 years, the immunity of vaccinated ewes was strong against oral challenge with the combined serotype I and V organisms given during advanced gestation, a period of high susceptibility to vibriosis infection. The 1968 results were inconclusive as a single abortion occurred in both the nonvaccinated and vaccinated ewes when their immunity was challenged. Factors to be considered include age resistance, exposure to infection, or reduced virulence of the original serotype I and V organisms in preparing the oral challenge inoculum.

2. Paratuberculosis. None

3. Respiratory diseases. Under a P.L. 480 grant at the College of Veterinary Science and Animal Husbandry, Mathura, India, studies are being conducted to determine the role of infectious or other agents in pulmonary adenomatosis and their relationship to the neoplastic nature of this disease complex.

The epizootiological, clinical, and pathological features of pulmonary adenomatosis are being studied at the Veterinary Department of the Institute of Agriculture in Titograd, Yugoslavia, under a P.L. 480 grant.

Research is beginning at the College of Veterinary Science of the Andhra Pradesh Agricultural University in Tirupati, India, under a P.L. 480 grant to isolate and identify the causative agents of pneumonia in sheep.

4. Foot rot. A cooperative project has been initiated at the University of California to study the pathogenesis of foot rot in sheep and to develop field methods for its control.

5. Bluetongue (BT). Bluetongue virus (BTV) has a complex morphology. The basic core particle has a diameter of 50-60 mμ. It has icosahedral symmetry with 92 subunits (capsomeres) along its crystalline faces. The capsomeres of the core particle are often obscured by a fine fibrillar meshwork which increases the total diameter of the particle to 60-80 mμ. Frequently, the core particle, with or without the fibrillar overlay, is surrounded by a unit membrane (envelope) of host cell origin. These membrane-bound structures may contain from 1-100 infectious particles.

It is known that BTV is very stable and that the virus will circulate along with specific neutralizing antibodies. The significance of the above data indicates that the host-specific cell membrane may isolate the viral particle from the inactivating influence of BTV-specific neutralizing antibodies. This study is important in determining the overwintering and reservoir of BT disease in the United States.

Bluetongue virus was titrated for the first time in embryonating chicken eggs (ECE) directly from the peripheral blood of BT-infected sheep. Three viral assay procedures were used for the experiments that covered 3 consecutive years. Virus was detected as early as the day after inoculation (DAI) 1 and as late as DAI 31 with the bulk concentration occurring from DAI 3 to 10. There was coexistence of BTV and its serum-neutralizing antibody in a high proportion of the infected sheep on DAI 21. The yolk-sac and intravascular routes of inoculating ECE were equal in virus detection, but the intravascular route was more sensitive.

A high titering viremia consistently occurred in cattle infected with BTV, either by artificial inoculation or the bites of BT-infected Culicoides variipennis. The virus was present in the blood as early as the 2nd day after infection, rose to a peak on the 7th day, and then gradually declined, but was still detectable as late as the 26th day. In two cattle tested on the 50th day, BTV was isolated from the blood via sheep inoculation, but could not be isolated in ECE. The cattle developed minimal clinical responses to BTV infection, and high levels of serum-neutralizing antibodies coexisted with virus in their blood.

Preliminary studies of BT in wildlife ruminants under a cooperative project at the University of Wisconsin, have been directed toward developing satisfactory laboratory procedures to study the virus, a limited serological study of the prevalence of the disease in the wild, and an investigation of the effect of the BTV vaccine on deer. After 5 months, satisfactory laboratory procedures have been developed to study this agent. Serological evidence suggests enzootic areas of BT in wild deer populations, and that deer are highly susceptible to BTV vaccine.

Lesions of lambs experimentally inoculated with contagious ecthyma virus progressed through macula, papula, vesicle, and pustule stages and finally scab formation. Granulomatous inflammation, pseudoepitheliomatous hyperplasia, and acanthosis were prominent lesions of the later stages of the disease. Histochemical, immunofluorescent, and electron microscopic studies revealed that the virus replicates at multiple sites within the cytoplasm of infected cells.

A field survey conducted under a cooperative project on viral ulcerative dermatosis at the Colorado State University Agricultural Experiment Station indicated that the disease incidence appears to follow a 2-year cycle.

Experimental reproduction of the disease showed the domination of bacterial infection. The virus isolation was not successful.

Studies are being done on the clinical characteristics in connection with the various factors of host-parasitism relationship. Last season, the external eyelid form and external genital form were found, but not the facial or leg form.

6. Foreign animal diseases. Wide variation in susceptibility of sheep to foot-and-mouth disease virus (FMDV) was observed in limited studies. Results varied from fatal infections to clinical signs so mild they might have been overlooked in a typical range situation. Viremia was demonstrated in all infected sheep from 1 to 4 days after exposure, and infectious FMDV was found in mucus samples from the esophagus and oropharynx long after clinical recovery from foot-and-mouth disease (FMD). Thus, the tendency toward inapparent FMD infections, especially in adult sheep, is compounded by a high conversion rate to the carrier state. A group of young, adult sheep was protected against virulent homotypic challenge 33 days after vaccination with a trivalent oil adjuvant FMD vaccine. After challenge inoculation, FMDV could not be recovered from the vaccinated sheep, while virus was easily recovered from the blood and esophageal mucus in all similarly exposed control sheep.

7. Sheep pox. Lyophilization studies of the virus of sheep pox are being conducted at the Faculty of Veterinary Medicine, Ankara, Turkey, under a P.L. 480 grant. Safety and potency tests indicate that a previously developed vaccine provides a good immunity. These researchers have demonstrated that immunity lasts at least 6 months and they plan to continue challenge studies for a longer period. Some field work with this vaccine has been undertaken.

Workers at the Institute of Veterinary Preventive Medicine, Ranipet, India, under a P.L. 480 grant, have determined the storage properties of an aluminum hydroxide-adsorbed sheep pox vaccine. Apparently these workers have the ability to produce a worthwhile immunizing agent. Their efforts have also been successful in cultivating the virus in cell cultures.

8. Listeriosis. Investigations of the epizootiology, pathogenesis, and prophylaxis of listeriosis in sheep and its importance in epidemiology in man have been initiated at the Research and Diagnostic Institute of the Veterinary Faculty, University of Sarajevo, Sarajevo, Yugoslavia, under a P.L. 480 research grant.

C. Poultry

1. Ornithosis. The bacteria that cause the psittacosis-trachoma group of diseases and that are assigned taxonomically to the genus Chlaymidia can be separated into two species. This separation is based on relatively stable morphological and chemical characteristics of the organisms rather

than on their presumed host or tissue preferences. The type species Chlamydia trachomatis is differentiated from the second species C. psittaci, by simple tests that can be performed readily in the laboratory. These tests are for glycogen production and sensitivity to sodium sulfadiazine.

The morphological and chemical characteristics of a group of 16 strains of chlamydiae of both species and representing isolates from turkeys, a pigeon, a sparrow, parakeets, a gull, a mouse, cattle, sheep, a muskrat, and a man, were tested. All strains fit the described patterns for taxonomic separation, and for the first time, species designations could be given these strains.

Polyarthritis caused by C. psittaci in approximately 50 of 2,000 feeder lambs in northern Iowa was studied. Clinical signs and gross and microscopic lesions were described. The causative agent was isolated, identified, and used to reproduce the disease in lambs. This is the first conclusive diagnosis of the disease in Iowa sheep.

2. Salmonellosis - paracolon infections. Studies of the formaldehyde (HCHO) concentration on and in chicken eggs following preincubation fumigation showed that the fumigant level was lower, but not significantly so, when eggs were stored at 10°C. after fumigation. After about 2 hours' storage at room temperature (24°C.), the concentration of HCHO on the eggs had dropped radically and by 21 days it was extremely low. Penetration into the shell membranes or the albumen of fumigated eggs was also very low. The bactericidal effects of formaldehyde fumigation have been much greater on the surface of brown-shelled eggs than white-shelled eggs, apparently because of differences in cuticular structure. After chemical removal of the outer egg covering, a very similar HCHO adsorption level and bactericidal effect were shown for both brown and white eggs.

The penetration pattern of Arizona bacteria through the outer structures of chicken eggs after 24 hours' incubation at 99°F. is very similar to that of Salmonella typhimurium. An average of 4.39 percent of exposed eggs revealed penetration of the Arizona bacteria to the inner surface of the inner shell membrane. These findings reemphasize the importance of producing eggs free of fecal contamination in preventing Arizona infections in poultry flocks. Studies of the penetration of S. typhimurium through the outer structures of turkey eggs showed that the thicker outer and inner shell membranes of these eggs render them more resistant to bacterial invasion. Chicken eggs that are cracked are readily penetrated by S. typhimurium at temperatures as low as 5°C.

To determine the cycle of Salmonella infections in turkeys, cooperative research was conducted at the University of Minnesota.

Two Salmonella serotypes, S. heidelberg and S. typhimurium, were studied in three turkey hatcheries. The studies on S. heidelberg indicated that, to

break the cycle of infection through the hatchery, Salmonella-free breeding flocks must be developed. The imported eggs for replacement breeders should come from Salmonella-free flocks.

An epidemiological investigation of S. typhimurium revealed that an infected breeder flock can infect other flocks on the same premises and lead to an explosive outbreak of S. typhimurium.

New serological methods to detect salmonellosis in turkeys are being studied to find better and more sensitive tests than those presently employed.

To break the cycle of infection, dipping of eggs in antibiotics is also being studied. In order to have infected eggs, a method of infecting the eggs was developed, and will be used to determine the effect of dipping eggs in antibiotics, and its effect on eliminating Salmonella from hatching eggs.

Eight bacteriophages were selected and used in a scheme to phage type the 712 isolates S. typhimurium, S. typhimurium var. cop., and S. heidelberg. Twenty-two S. heidelberg, and 26 S. typhimurium phage-typing schemes were demonstrated.

In the cooperative work at the University of Minnesota, studies on Arizona infection in two turkey hatcheries were continued.

One hatchery and breeder flocks on three farms remained free of Arizona infection through the monitoring programs involving rectal swabs, blood testing, and cull poults over a 3-year period. Arizona-free breeder replacement poults are the key to the success.

In the second hatchery, Arizona infection was widely distributed in poult replacements for breeder flocks and inadequate cleanup on the breeder farms compounded the problem. Day-old poult inoculation with antibiotics keeps the losses in poults to a minimum but is not a long range solution of the problem.

The third vaccination experiment of breeder birds with a commercial bacterin indicated no value in protecting the birds from infection and egg transmission. Furazolidone had little value in protecting breeder hens from infection.

The indirect hemagglutination test was more sensitive than the standard tube test in detecting Arizona carriers but chronic carrier birds were missed.

Under a P.L. 480 grant to the Department of Veterinary Bacteriology and Hygiene of Punjab Agricultural University, Hissar, India, a study of Salmonella infections in domestic animals has been initiated. The chief objective of this research is to isolate sources of specific types of this disease and to determine how it is transmitted by both animals and man.

The initial stage of this study was concerned with the sources of infection in animals. Out of the 914 adult birds, 29 eggs, and 7 rats examined on a poultry farm, 20 birds and 1 rat had Salmonella. In addition, fecal examination of 291 buffalo calves and 284 zebra calves yielded 10 and 3 Salmonella isolates, respectively. Serological typing of isolates is being done simultaneously.

3. Respiratory diseases of poultry. A hemagglutination-inhibition (H-I) test to demonstrate antibodies against Mycoplasma meleagridis has been developed. Antigen used in the test was prepared from a hemagglutinating strain of M. meleagridis isolated from a turkey embryo. Examination of serums from both naturally- and experimentally-infected turkeys using this test gave results similar to those obtained with a tube agglutination test. The results indicated that the H-I test is effective to show M. meleagridis antibody response in turkeys. The guanine-cytosine (GC) base compositions of the deoxyribonucleic acids of strains of Mycoplasma of avian origin and some from other sources were determined from buoyant density in CaCl. The values ranged from 24 to 35.7 mole percent, and four groups with modal values of 24, 27, 32, and 35 percent were derived. The 27 percent group included the values from 26 to 30 and may comprise more than one group. A fifth group of M. pneumoniae with GC base composition of 39 percent was recognized from the literature.

In cooperative research at the University of Minnesota, epidemiological investigations were continued on all known and suspected cases of infectious sinusitis.

The results of an intensified control program for infectious sinusitis indicate that the level of infection is very low, and that it is possible to eradicate M. gallisepticum from turkey flocks. However, to realize this result, the infection in chickens must be eliminated, and safeguards against contaminated live virus infections provided.

The cycle of M. meleagridis infection is primarily due to seeding of the oviduct by contaminated semen during the insemination process. Results indicate that the cycle can be broken by using noncontaminated semen and by egg dipping.

At the University of Wisconsin Agricultural Experiment Station, a micro-complement fixation (CF) test was perfected for use in serotyping laboratory strains of avian Mycoplasma. It was further used to aid in identifying and characterizing Mycoplasma isolated from wild turkeys in Texas and Wisconsin.

Several isolates of Mycoplasma have been recovered from the tracheae of wild turkeys trapped in Texas at the Welder Wildlife Refuge and from wild turkeys killed in Wisconsin during the annual spring hunting season. These isolates are unique in that they are able to form well-developed colonies on solid mediums within 18 hours. These isolates were tested by the micro-CF method

against 16 "known" antiserums to avian Mycoplasma. There was no significant fixation of complement by any of the antiserums. In addition, the Ouchterlony technique of double diffusion is being modified for use in studying antigenic relationships between strains of avian Mycoplasmas isolated from chickens and turkeys.

Cell cultured-propagated strains of infectious bronchitis virus (IBV) are being evaluated in neonatal and young adult chickens to determine their antigenicity, pathogenicity, and cross protection. Preliminary results show the production of high levels of neutralizing antibody capable of protecting birds against subsequent challenge inoculation with virulent strains of IBV for at least 15 weeks.

Fluorescein isothiocyanate-labeled antiserums have been used to detect intracellular IBV in cell cultures from heterologous donors. These cell systems produce IBV of varying titers as determined by titration of cultural fluids in chick embryo kidney cell cultures.

A study was continued on the antigen-antibody response produced by the inactivated Newcastle disease vaccine. Relatively little progress has been made in the past in efforts to cause a passive immune body (system) to produce antibodies when inoculated with the homologous antigen. The existence of humoral or cellular antibodies (passive or active) usually directly or indirectly degrades the antigen and often results in lower testable titers and resistance of the host. If the antigen is able to survive destruction and the antibody level falls to an insignificant level (near susceptible), and if the antigen can become active, there is a lag phase of approximately 7 days of high susceptibility. This is followed slowly by new antibody production as evidenced by increasing livability (survival) following challenge inoculation with virulent homologous virus. A vaccine with an acceptable level of competence has been prepared that stimulates satisfactory protection at all intervals postvaccination for 10 to 12 weeks. The vaccine contains inactivated Newcastle disease virus antigen complexed with the homologous antibody mixed with an aluminum hydroxide gel adjuvant.

A new procedure for utilizing alum as an adjuvant in preparing vaccines has provided products that stimulated excellent protection in susceptible chickens.

Oral administration of an inactivated Newcastle disease vaccine resulted in production of adequate levels of immunity in vaccinated chickens.

The cooperative study at the University of Wisconsin Agricultural Experiment Station was concerned with the development of a medium on which lentogenic Newcastle disease strains will form plaques. These strains, widely employed as vaccines, require the presence of magnesium and DEAE dextran. Use of mediums both with and without these additives makes it

possible to monitor the purity of seed stocks used in vaccine production. It also makes it possible to directly study population of vaccine viruses.

Further investigation of wild-type viruses gives further support to the hypothesis that these strains are heterogeneous and usually contain a family of related mutants.

Intensive study of one wild strain has revealed six plaque types from which clones can be derived. Three of these are clear plaques of differing sizes which do not give rise to further mutants. Three are red plaques, also of differing sizes, which have a rather high mutation rate to clear. A tiny red plaque appears to be the stem virus. The clones differ in virulence, two as mild as vaccine strains and one as lethal as any known velogenic strain. Growth rate, enzymatic activity, antigenicity, and physical stability of these clones also differ.

These findings make it necessary to reexamine studies on induction of mutants, on methods used to isolate virus, and also our concepts of the epizootiology of Newcastle disease. All known kinds of mutants were selected from this strain without use of mutagens. Isolation procedures were selective, often picking up only a portion of the populations. Because of persistence of virus in immune hosts and presence of variants differing in antigenic pattern and virulence within a single isolate, many of our current assumptions concerning the spread and control of NDV are questioned.

The study of the egg transmission of M. gallisepticum was concerned with breaking the transmission cycle. For this purpose, normal eggs from hens that were artifically infected with M. gallisepticum were divided daily into heated and control groups. Those eggs to be heated were placed in an incubator with a total load of 2,000 eggs. It required 11 to 13 hours for the heated eggs to attain internal temperatures of 112 to 115°F. Eggs reaching approximately 113.5°F. or more contained no viable M. gallisepticum when cultured at the 14th day of normal incubation, as compared to proved infected control eggs. Control eggs heated under the same conditions had a 10 to 15 percent reduction in hatchability. The heating procedure should offer a practical means of breaking the egg transmission cycle of M. gallisepticum in primary breeding stock where the moderate reduction of hatchability can be tolerated.

Normal White Leghorn hatching eggs were heated at varying temperatures for 10 to 15 hours just before being incubated under normal conditions for hatching. Two thousand eggs at room temperature (approximately 76°F.) required 13 to 15 hours to attain internal egg temperatures as high as 114 to 116°F.

Various combinations of medium ingredients were studied to devise a more adequate medium for the isolation and cultivation of most serotypes of avian Mycoplasma. Swine and turkey serums were best for enrichment of mediums containing both large and small polypeptide protein bases.

The infectious bronchitis-Newcastle disease virus (IB-NDV) interference phenomenon in cell cultures was successfully used to quantitate IBV antibody levels in serum. Three serological types of IBV were distinguished from each other by immune serums using the IB-NDV test. Slight "one-way" cross reactions occurred between IBV antiserums. Using this test to study serological relationships among IBV strains and to measure IBV antibody levels should prove useful in attaining immediate and long-range research goals. The test provides considerable savings in time and labor with nonembryo-lethal IBV strains.

The measurement of Newcastle disease virus infectivity was made with bits of viable embryonic membranes still attached to fragments of egg shells. The basic technique was modified to utilize simple equipment and is a rapid, accurate measure of virus and neutralizing antibodies. It should be beneficial to some diagnostic and many research laboratories.

By doing a complete virus titration on bits from a single egg, eggs from parents genetically selected for resistance or susceptibility to disease were compared. No dependable differences were demonstrated that paralleled challenge results in chicks.

Results indicate differences in protection afforded broilers against disease by combination infectious bronchitis-Newcastle disease drinking water vaccines. Challenge techniques and protection criteria were kept realistic to provide information applicable to broiler-growing situations.

Five isolates of IBV have been obtained that show significant differences from prototype bronchitis viruses when examined by cross-serum neutralization procedures. Additional data are needed to determine if these are prime strains or represent new serological types. Preliminary data indicate that these differences also are demonstrable by cross-challenge tests in chickens. The poultry vaccination program would have to be reexamined if a multiplicity of strains exists in the field, or if significant antigenic drift occurs with the infectious bronchitis virus.

Infectious bronchitis virus is inactivated by N-acetylaziridine at 37°C. in 4 hours. This is true for four strains, Mass., Conn., Iowa 97, and Delaware JKM. Antigenic capabilities of these inactivated viruses are presently being examined in chickens, the objective being an inactivated vaccine for IBV.

In another study, M. synoviae hemagglutinating (HA) antigen has been produced that agglutinated chicken and turkey red blood cells. The progress of this work to date shows that M. synoviae and M. gallisepticum infections in

chickens can be differentiated by testing the serum with M. gallisepticum HA and the experimental M. synoviae HA antigen. Cross-reaction to both antigens occurs infrequently, but the homologous serum reacts at a higher dilution than the heterologous.

While M. gallisepticum serum plate, tube agglutination, and HA antigens are available for official testing of chickens and turkeys, there are no M. synoviae antigens commercially available. There is no official M. synoviae test.

Nearly all turkey flocks are free of M. gallisepticum, but some flocks occasionally have problems with M. synoviae and M. meleagridis infections. If the M. synoviae HA antigen can be used in diagnosing M. synoviae infection in turkeys, it will be of significant help in producing flocks free of Mycoplasma infections.

To study susceptibility of chickens to respiratory infections in controlled environments it was necessary to design and fabricate environmental cabinets especially suited for chickens before research could begin on the importance of the environment in the health of poultry. The cabinets have floors approximately 4' x 6'. The temperature, relative humidity, and air exchange rates are regulated by template controllers. Studies are in progress on the influence of cyclic and constant environmental temperatures on broiler growth.

Cabinets especially designed to study the influence of controlled environmental conditions on disease transmission were constructed. Experiments will begin in these newer units when the air conditioning control equipment is completed.

Poultry production buildings were modified so that all air is brought in by fan through a filter medium. The filtered air prevents the entrance of airborne particulate that may contain disease-producing microorganisms. Relief valves assure that the buildings are under positive pressure relative to the outside air, thus preventing the leakage of unfiltered air into the house. The laboratory research program requires that disease-free flocks be maintained near those flocks that are diseased. This has been accomplished by the positive pressure-filtered air poultry house system. The knowledge gained in this field could have important practical application at the primary and replacement poultry breeder area.

A study was conducted on turkey influenza virus at the University of Wisconsin Agricultural Experiment Station.

Various biological characteristics of the influenza A/turkey/Wis/66 virus were studied. The virus grew well under a variety of conditions in embryonated eggs but not in tissue culture systems. The virus is very stable and allantoic fluids lose very little infectivity after 1 week at room temperature. Environmental factors affected the course and character

of infection and disease in turkeys of various ages. There was also considerable variation in the clinical signs of disease. Virus could be isolated from tracheal swabs while birds had low levels of circulating serum antibody. Apparently recovered birds, when subjected to stress by chilling, had persistent infection as measured by further isolation of virus and increases in level of antibody. Serological data suggested that virus persisted in some birds for long periods of time and would be released to stimulate immune competent cells upon cold stress. Low levels of antibody, which persisted over long periods of time, were common. In pathogenesis studies, virus was disseminated rapidly throughout the body and was isolated from heart, lung, trachea, gonad, spleen, and kidney tissue. Virus could not be isolated from blood. Pheasants, ducks, and domestic geese were infected and could transmit the virus by contact with susceptible birds.

Research was conducted also in the area of designing equipment for greater laboratory and animal room safety.

Sterilization of equipment sensitive to heat was effectively accomplished with ethylene oxide (ETO). Safe blends of ETO and Freon 12 are routinely used. Commercially available equipment for this purpose is complex and extremely expensive. Small table top models are available but these limit the sterilizable equipment to small items.

With this problem in mind, an ETO sterilizer was developed to accommodate long pieces of equipment such as embolectomy catheters (36"). A 40-inch piece of steel pipe, to which a "hand hole" cover was attached for access, formed the body of the sterilizer. Legs, gas ports and valves, a pressure-vacuum gauge, and a heating element were attached. The body of the sterilizer was insulated with fiber glass and covered with a light gauge stainless steel.

The total cost of this unit, including parts and labor, was under $200. Working drawings have been made and are available upon request. An article for publication is in progress.

For some time, it has been suspected that high pressure cleaning devices create hazardous aerosols when used to clean contaminated surfaces. To determine if this hazard exists, a number of microorganisms was incorporated into a series of artificial soils which were painted on wall surfaces and dried. Several different detergent-disinfectant solutions were applied to the wall surface with a high-pressure sprayer to remove the soil. Simultaneously with, and a few minutes after cleaning, samples of the air in the room were collected. These samples were assayed for viable microorganisms.

The number and size of the particles containing the organisms indicate that cleaning procedures produce bioaerosols of suitable size to remain airborne indefinitely. These might cause infections if the organisms contained in the air were pathogenic and were breathed into the respiratory system of susceptible men or laboratory animals. An aerosol produced in this manner

could also contaminate nearby equipment and other materials. The aerosol may also be conveyed by air movement to other areas where it would continue to be a hazard.

Wide variations were noted in the survival of organisms in the air during the cleaning procedure when different chemical agents were applied to the soil. Survival of the different test organisms also varied widely when subjected to the same chemical treatment. Effective typical disinfectants are not necessarily effective in reducing the number of airborne organisms.

In cooperative study at the University of Maine, Orono, the specific pathogen-free (SPF) progress for broilers and breeding hens has been further evaluated since its initiation in 1961.

During this period, 15,068,040 broilers were monitored for respiratory diseases and condemnations at the processing plant. Through a system of improved management practices, and the use of killed Newcastle and other inactivated vaccines, together with accurate monitoring of diseases through laboratory diagnosis, it has been established that M. gallisepticum and Newcastle disease can be eradicated.

During the past year, a concentrated effort has been made to resolve the epizootiology of infectious bronchitis. Marek's disease and synovitis (M. synoviae) have also received special attention relative to transmission and control.

Under a P.L. 480 grant to the Hebrew University, Hadassah Medical School, Jerusalem, Israel, research on the structure, chemical composition, immunochemistry, and nutritional requirements of PPLO (Mycoplasma) pathogenic to farm animals has produced the following results.

A method for identifying Mycoplasma and other microorganisms by polyacrylamide-gel electrophoresis of cell proteins has been developed and used successfully in solving several taxonomic problems. This method showed conclusively the identity of the Negroni agent isolated from tissue cultures with M. pulmonis, the identity of M. hominis Type 2 with M. arthritidis, and the identity of Tumor 7 strain isolated by Dr. Sabin with M. hyorhinis. The method enabled the rapid identification of fresh isolates obtained from man, dogs, and tissue cultures. Furthermore, preliminary results indicated its applicability to other bacteria. The method is now being used for classifying Mycoplasma isolated from various farm animals.

Progress was made in the study of membrane reformation from disaggregated Mycoplasma membranes. Plasma membranes of M. laidlawii and M. gallisepticum were solubilized by several ionic and nonionic detergents. Solubilization of the membranes by sodium dodecyl sulfate (SDS) separated membrane lipid from protein as demonstrated by polyacrylamide-gel electrophoresis of the solubilized material.

The solubilized membrane material reaggregated spontaneously on removal of the detergent by dialysis or by Sephadex G-25, forming vesicles limited by a triple-layered membrane of about the same thickness as the original Mycoplasma membrane. A divalent cation (e.g., Mg^{2+}) was essential for membrane reassembly. The ratio of lipid to protein in membrane reaggregates varied considerably according to the Mg^{2+} concentration. At a low Mg^{2+} concentration, reaggregates contained a higher percentage of lipid. The present results bear out the suggestion that the SDS-solubilized membrane material does not consist of homogenous lipoprotein subunits, but of separate SDS-lipid and SDS-protein complexes. The reassembly of solubilized membrane lipid and protein, on removal of the detergent, indicates that these components contain sufficent structure-determining information to interact spontaneously in the presence of Mg^{2+} and produce membranous structures.

Preliminary results show that the solubilization of Mycoplasma membranes by sodium dodecyl sulfate does not destroy their antigenic properties, enabling the immunological characterization of membrane proteins.

The changeability of biological properties of viruses and the relationship between hosts and viruses have been studied under a P.L. 480 grant at the Veterinary Research Institute, Pulawy, Poland. The pathogenic strain of Newcastle disease virus (NDV), (Radom B), and nonpathogenic strain (Roakin) were passaged 30 times in embryonating chicken eggs. Although the study has not been completed, there is adequate evidence indicating a decrease in virulence of the Roakin strain of NDV when subjected to various temperatures of hatching chick embryos.

The effects of prolonged feeding terephthalic acid (TPA) to rats have been investigated under a P.L. 480 grant to the Hebrew University, Hadassah Medical School, Jerusalem, Israel.

Additional observations have been made in rats fed various levels of TPA. There is further proof of growth depression in both male and female animals after feeding TPA. The toxicity of the 5 percent diet is further confirmed; 33 percent of the animals on this level have died. On the average, death occurs after 230 days' feeding in the males, and 170 days' feeding in the females.

The consistent pathological findings are stones in the urinary tract with obstruction and focal lesions in the lungs. Microscopically these findings are manifested as pyelonephritis with hyperplasia of the pelvic and bladder epithelium. In two cases, definite transitional cell papillomas of the bladder were found. In the lung, the finding was a focal interstitial pneumonitis. One animal on 1 percent TPA had a large subcutaneous tumor which appeared fatty with nests of suspicious cells.

4. Bluecomb of turkeys. In the cooperative work at the University of Minnesota, the study concerned the microbiological, physiological, and related aspects of bluecomb disease.

Suspensions of intestinal tracts taken from turkeys from various bluecomb disease outbreaks were passed through chromatographic columns. Selected fractions of the eluates from these columns were injected into turkey poults and inoculated onto various types of cell cultures. Freezing as a method of preserving pathogenicity of column eluates was also studied.

Poults inoculated with one specific fraction of the fresh column eluate had severe signs of bluecomb disease and recovered poults were resistant to subsequent challenge inoculation with pathogenic intestinal suspensions. Viruses were isolated in chicken embryo cell cultures, from two specific consecutive fractions of the column eluates. However, such viruses were not pathogenic for poults.

Electron microscopic studies were done on pathogenic fractions of column eluates, on infected chicken embryo kidney cells, and on contents from the ceca of turkey poults that died from bluecomb disease. Papovavirus-like particles were found in the column eluate fractions and in cell cultures inoculated with that column fraction. Reovirus and enterovirus were found in cell cultures inoculated with fractions from other column eluates.

The pathogenicity of intestinal filtrates for turkey poults was destroyed when such filtrates were subjected to 4.8 percent chloroform (10 minutes at 4°C.) and heat at 50°C. for one hour with or without the addition of $MgCl_2$. The pathogenicity was also destroyed by changing the pH of the filtrate to 3.0 and letting the filtrates stand for 30 minutes at room temperature. The pathogenic agent did pass a 100 mμ millipore filter. These findings suggest that the agent is viral, labile, and smaller than 100 mμ.

In addition, cell cultures made from whole chicken and turkey embryo, chicken and turkey embryo kidney, and from turkey intestine were inoculated with pathogenic intestinal filtrates made from turkeys sick with bluecomb disease. Cytopathogenic viruses were isolated in all five cell types. However, chicken embryo kidney yielded the highest number of isolations.

Intestinal filtrates made from 13 geographically widely separated bluecomb outbreaks were collected and tested. Virus isolates were made from nine of these 13 filtrates. Five of the nine isolates were selected and fully characterized and classified into proper virus groups. Three of five isolates were enteroviruses, one was reovirus, and the fifth isolate was a papovavirus. This is the first report of a papovavirus isolated from the avian species. None of the cell culture isolates were pathogenic for day-old turkey poults and inoculated poults showed no resistance to challenge inoculation with pathogenic bluecomb intestinal suspension.

Maintenance of bluecomb seed material at a consistently pathogenic level can best be accomplished by continued serial poult passage at three- to four-day intervals.

Characterization of bacteria of the genus Vibrio isolated from the intestinal tract of turkeys sick with bluecomb disease was completed and a comparison made to Vibrio in other animals and man. Turkey intestinal Vibrio were different in some characteristics from any of the other Vibrio with which they were compared.

The physiological investigations were designed to determine the pathogenesis of bluecomb disease and methods for treating sick turkeys.

Results obtained from measuring several parameters indicate that many of the signs of bluecomb disease are associated with abnormal physiology of the digestive tract leading to loss of appetite and weight. Starvation, lowering of body temperature, wet litter, and reduced motility of the digestive tract contribute to the general debilitation.

It is postulated, from results obtained, that the infective material contains agents that cause abnormal gastrointestinal function, thereby inhibiting eating and causing signs of the disease. Food present in the digestive tract may not be properly digested and absorbed during the disease. Therefore, force-feeding or liquid diets may have limited value as therapeutic measures.

The electron microscopic investigations revealed no characteristic particles representing any of the known animal viruses found in the bird's cecal contents. Eight morphologically distinct bacteriophages were present. Of these, three were new types of bacterial viruses. Control cecal contents of uninfected turkeys contained fewer bacteriophages. This observation suggests that some consideration might be given to the possibility that bluecomb disease is the result of an imbalance in microbial fauna because of infection with spurious bacteriophages.

In cooperative research at the Texas Agricultural Experiment Station, College Station, Texas, preliminary characterization studies revealed the bluecomb agent to be ether- and chloroform-susceptible and capable of passing 0.2 micron filters without loss of infectivity. Rapid serial passage of infectious material in day-old poults is apparently the only dependable way to keep the agent virulent. Although the bluecomb agent was apparently grown in embryonating eggs and in cell culture, virulence of the agent(s) for turkeys was lost by such procedures. Efforts to produce bluecomb with Truscott's Vibrio were unsuccessful.

In cooperative research conducted at the University of Wisconsin, attempts were made to isolate the causative agent of bluecomb disease. For this purpose, several virus isolates have been obtained from the intestinal tracts of turkeys with clinical signs and postmortem lesions of bluecomb

disease. These virus isolates have been identified as belonging in the reovirus group based on virological and serological characteristics.

The results of inoculation and immunoserological studies suggest that these viruses are involved in the bluecomb syndrome of turkeys.

5. Hemorrhagic enteritis in turkeys. The study of this disease conducted in cooperative agreement with the Virginia Polytechnic Institute, Blacksburg, Virginia, included 37 experiments with 1,287 turkeys.

It resulted in development of a selective solid medium for isolation and tentative identification of fecal Streptococci. Fecal Streptococci have been assayed for bacteriocene production and susceptibility.

The primary agent is a rather small virus, relatively resistant to heat. Also, there is considerable evidence that other factors are involved. The agent is widespread in its distribution in the U.S. but losses may vary widely from area to area and from flock to flock. Convalescent serum will greatly reduce losses when given intramuscularly at the onset of mortality. Serum does not protect against simultaneous intravenous challenge inoculation but does inactivate virus when serum and virus are incubated together before challenge inoculation. The disease was not produced in turkeys less than 5 weeks old and susceptibility increased through 6 to 10 weeks of age. Pathogenicity can be increased by injecting certain streptococcal materials from 1 to 21 days before challenge. Attempts to produce a satisfactory vaccine, although promising, are not as yet successful. The agent was found in serum, liver, and spleen of infected turkeys.

Inoculation of turkey embryos by various routes and at various periods of embryonation resulted in no specific pattern of lesions or mortality. Material recovered from the embryos did not prove infectious for young turkeys.

6. Duck virus enteritis (plague). A seed virus for preparing vaccines was developed and delivered to the Eastport, Long Island Duck Research Laboratory. Examination of 8,412 serums from commercial duck flocks, migratory waterfowl, and aviaries from 24 states indicated that the disease incidence is limited to New York State. Duck virus enteritis (DVE)-attenuated virus was inactivated in $3\frac{1}{2}$ hours by 0.05 1-acetylazíridine. Five preparations of chicken embryo-adapted DVE virus were compared as to their serological and immunological response in 2-week-old ducklings. Three preparations, two of which had been inactivated at 6 and 24 hours, were administered intramuscularly. Two other preparations were given orally, one of which was a cell culture-propagated virus. The serological and immune responses were higher with intramuscularly inoculated preparations than with those orally-administered.

D. Swine

1. Pathogenesis of brucellosis in swine. A study of the characteristics, distribution, and frequency of microscopic lesions from Brucella suis in male swine showed that the lesions are granulomatous. Changes were observed most frequently in lymph nodes, accessory genital organs, livers, and bones (in that order). The most common sites for recovery of Br. suis were determined by bacteriological examination of 147 infected swine. Lymph nodes were the most frequent source of Br. suis. Genital organs, spleen, liver, and blood were also frequent sites of recovery. The number and variety of locations from which organisms were recovered indicated the widespread distribution of Br. suis in infected swine. However, 91 percent of the swine would have been identified as infected by culturing the mandibular, suprapharyngeal, gastrohepatic, and internal iliac lymph nodes only. In a review of data on reproductive failure from swine brucellosis, it was concluded that embryonic death and fetal abnormalities do not result from Br. suis infection. Abortion is the most serious complication of brucellosis in female swine, but the majority of pregnant swine with brucellosis do not abort. Abortion is influenced more by the time of exposure than by the stage of gestation.

2. Hog cholera. A serum neutralization, fluorescent antibody, cell culture system proved that 72 of 90 swine (80 percent) had been exposed to hog cholera virus (HCV), whereas exposure was proved in only 63 of 90 swine (70 percent) by in vivo, sublethal virus challenge inoculation. The greater sensitivity of the serum neutralization test in revealing the presence of HCV was confirmed by more severe clinical reactions in pigs exposed to the serum neutralization test than in pigs exposed by challenge inoculation.

Hog cholera virus was partially purified using a chromatographic procedure with magnetic ferric oxide. Not more than 50 percent of HCV infectivity was lost concomitant with 90-95 percent reduction of extraneous organic nitrogen. Electron microscopy of negatively-stained samples of concentrated infective HCV revealed 40 to 50 mμ virus-like particles and a large number of 12-15 mμ entities. The 40-50 mμ particles were surrounded by a poorly-defined, asymmetrically-arranged, sac-like membrane or appendage.

Cultured horse kidney and skin cells were not susceptible to HCV. Neither X-ray treated nor nontreated hamsters developed tumors when inoculated with PK-15 cells. Labeling swine kidney cells with radioactive thymidine revealed labeling of both "sides" of the chromosomes at the first mitosis but on only one side after the second mitosis. Interaction of two swine kidney cell lines did not produce a hybrid cell but instead, one displaced the other. Good preparations of chromosomes were obtained from horse kidney cultures.

Swine may carry and disseminate HCV for several months before they succumb to the cumulative effects of the disease. Coincident with an apparent

immune response by affected swine, virus in serum was occasionally masked by antibody or inhibitor or both. Thus, several samples of serum, collected at intervals, were necessary to confirm the presence of virus.

In cooperative studies at the University of Nebraska, the work was concerned with the effectiveness of the Nebraska fluorescent antibody test (NFAT) for detecting modified live virus (MLV) in infected pigs. In general, the results of NFAT coincided with the histological findings. This test may be helpful in identifying the less apparent outbreaks of hog cholera.

3. Swine erysipelas. Techniques have been developed for use in investigating the behavior of Erysipelothrix rhusiopathiae in soil. The antibiotic medium previously described was satisfactory for detecting very small numbers of the organism in soil. A method also was developed for the enumeration of viable cells of the organism in soil, using a modification of the most-probable-number technique.

The pathways of glucose catabolism in Ery. rhusiopathiae were identified using the radiorespirometric technique. Data showed that 96 percent of the glucose catabolism was via the Emden-Meyerhof-Parnas pathway with the remaining 4 percent dissimilated by the hexose monophosphate pathway. The products of the anaerobic dissimilation of glucose were lactic acid, the major product, and ethanol, acetic acid, formic acid, and carbon dioxide in smaller amounts.

4. Abscesses. Two bacterins were tested in pigs: (a) cells of Lancefield's serologic group E Streptococci, and (b) the same strain of Streptococci but with the addition of concentrated cell-free culture filtrate (CCF). The CCF was prepared from the supernatant medium after harvesting the cells for the bacterin. Each preparation was incorporated in an equal amount of Freund's adjuvant.

The pigs in groups A and B were given subcutaneously 4 ml. of bacterins A and B, respectively. A third group (C) served as nonvaccinated and exposure controls.

All pigs were exposed on day 28 after vaccination by adding to their pelleted feed ration a beef infusion broth culture of the homologous strain of group E Streptococci.

When the experiment was terminated about 6 weeks after exposure, abscesses were found in 11 of 13 pigs in group A, in 14 of 14 pigs in group B, and in 13 of 14 pigs in group C. Group E Streptococci were recovered from the abscesses examined.

Under the conditions of this experiment, the results give no encouragement to the use of a bacterin for controlling abscesses from group E Streptococci.

In cooperative study of this disease at the Colorado State University, Fort Collins, Colorado, five intact male shoats acquired infection with Streptococcus suis and developed jowl abscesses as readily as five recently castrated male penmates. A castrated male hog that had abscesses and regained health carried Str. suis (positive tonsil swabs) up to the time of this report (15 months postinfection). When this hog was scrubbed with roccal disinfectant and placed in a disinfected room in contact with healthy pigs, they (21 total) became sick within 5 to 9 days and all had jowl abscesses. These 21 pigs were divided into 3 groups and placed in contact with the carrier at 2, 10, and 14 months of the 15-month interval. Streptococcus suis was recovered from the pigs' drinking water.

Thirty pigs were used to determine the early pathogenesis of jowl abscess; i.e., streptococcic lymphadenitis of swine (SLS). The pigs were infected orally (Str. suis culture in feed) and one was necropsied at 6 hours post-infection and at each 6-hr. interval thereafter through 120 hours. Febrile reactions and neutrophilic leukocytosis occurred in 30 to 48 hours post-infection. Tremendous numbers (10^{11}) of Str. suis per gram of palatine tonsil were recovered, but tonsil was little affected. Mandibular lymph nodes were invaded within a few hours with petechial hemorrhages in the lymph sinuses as early as 12 hours postinfection in some pigs. Progressive necrosis and purulent exudate in the lymph sinuses were observed grossly from 60 to 120 hours postinfection.

The research conducted on a cooperative basis at the Purdue University, School of Veterinary Science and Medicine, was designed to provide knowledge leading to methods to control cervical lymphadenitis of swine (jowl abscess).

Etiologic studies revealed that Streptococcus group E, serotype IV was the principal (if not the only) cause of the natural disease. It was the most common bacterial species recovered from affected swine and it caused an experimental disease indistinguishable from the natural condition when administered intranasally to swine of different ages (5 weeks to 2 years). The minimum number of bacteria required (MID_{50}) was $10^{5.8}$

Factors associated with virulence that were elaborated by Streptococcus group E included capsules, streptokinase (an enzyme that lyses plasma clots), streptodornase (deoxyribonuclease), and streptolysin O (an oxygen-labile hemolysin). The group and type specific antigens of Streptococcus group E could be separated and purified by electrophoresis, gel filtration, and ion exchange chromatography.

The cooperative research at the University of Missouri, School of Veterinary Medicine, Columbia, Missouri, was designed to study the relationship between the quantity of inoculum given in feed and the incidence of abscesses in the body.

There appeared to be no relationship between the quantity of the infective agent administered orally in the feed and the number of pigs having one or more abscesses in the jowl region. A pig needed to consume only a few of the infective bacteria to develop jowl abscesses, but a more efficient method, other than quantity, was needed to produce a 100 percent incidence.

In addition, it has been shown that a regular bacterin was of little value, but that some protection to jowl abscesses could be instigated with repeated inoculations and the use of materials to enhance the effectiveness of the bacterins.

5. Transmissible gastroenteritis (TGE). When pregnant sows were inoculated with a bacteria-free suspension of TGE-infected tissue from baby pigs, they were immunized so that they protected their baby pigs from an exposure to TGE-infected intestinal tissue. There was a good antibody titer in the milk and serum against the cytopathogenic virus commonly isolated on cell culture from TGE-infected tissues of baby pigs. Although there is a good antibody response following injection of cell-cultured virus into pregnant sows, they would not protect their pigs from an exposure to TGE-infected tissue.

Comparative studies showed that several strains of canine distemper, canine hepatitis, and measles viruses will cause TGE in baby pigs. They also showed that survivors are resistant to a further exposure of TGE virus from baby pigs, although there were no circulating antibodies to the cytopathogenic virus commonly isolated from TGE on cell culture. Fluorescein-conjugated TGE antiserums will fluoresce with canine infectious hepatitis-infected cells, but not with canine distemper-infected cells. Adenoviruses have been isolated from TGE-infected pig tissues.

The "modified" strain of hog cholera virus (MHCV) was concentrated and partially purified by centrifugal procedures. Characteristic particles were then identified by direct immuno-electron microscopy. The MHCV virion is a 40-50 mμ membrane-bounded flaccid particle which bears 12-15 mμ shear-sensitive surface projections. The latter represent its "soluble antigen." This work represents the first successful attempt to utilize the method of direct immuno-electron microscopy for identifying the virions and other virus-specific particles of a previously uncharacterized agent. Similar HCV characteristic particles were subsequently identified in the Monovet strain of HCV after concentration and partial purification by alternative methods. These observations should make possible large-scale production of noninfectious vaccine antigen.

Three size ranges of characteristic particles were identified in bovine viral diarrhea (BVD) by direct immuno-electron microscopy. The BVD virions are variable in size (80-100 mμ) and have no distinctive morphological features that are useful for identification. Smaller particles, 30-40 mμ, are viral degradation products. The BVD "soluble antigen" is

15-20 mμ, somewhat larger than that of HCV, and is remarkably similar in appearance to ribosomal particles.

Direct immuno-electron microscopy of two commonly encountered cytopathogenic TGE isolates showed that they do not share a common antigen. Morphologically, these agents are readily distinguishable; i.e., one is 25-30 mμ and is similar to the enteroviruses while the other agent is 70-100 mμ and resembles the virion of infectious bronchitis virus. The morphology of the latter agent is altered on storage in infected cell cultures. The significance of this alteration is under investigation. A number of adenoviruses are being investigated to determine if any of their virus-specific products bear any relationship to TGE.

Morphological and histochemical examinations of TGE in baby pigs showed that the cellular alterations were confined to the villus cells of the jejunum. Accelerated destruction and improper maturation of jejunal villus cells, resulting from virus replication, produced villus atrophy that was responsible for the clinical syndrome.

In cooperative research at the University of California Agricultural Experiment Station, an early diagnosis of TGE could be made using the fluorescent antibody technique when applied to smears of tissues removed from the tonsils or intestinal tract of experimentally-infected pigs. The method was specific and sensitive, permitting identification of the virus during the first few days of illness. The applicability of this test with outbreaks of the disease under field conditions has not yet been evaluated.

Two additional viral agents have been isolated from swine which are serologically related to other known enteroviruses but do not cause TGE. The presence of these agents as a mixed infection with TGE virus may complicate diagnosis of the latter since they both cause similar reactions in tissue cultures employed in diagnostic procedures.

6. Atrophic rhinitis (AR). The specific etiological agent has not been identified. However, atrophy of turbinates of susceptible swine can be reproduced by contact exposure (susceptible/infected) as well as by nasal instillation of nasal tissue homogenates and/or nasal saline washings from adult carrier swine.

Serial dilution of the inoculum reduced the number of positive transmissions, indicating that AR is caused by transmissible agent(s).

Transmission experiments did not have any correlation between quantity of calcium-phosphorus in the ration and the severity of turbinate atrophy present.

Individual bacteria isolated from AR crude transmissible material did not produce AR when used in susceptible pigs, suggestive of viral involvement.

Hysterectomy-derived, specific pathogen-free (SPF) 5-day-old pigs were highly susceptible to common bacteria in the fresh, unfrozen infectious material and died before the time required for atrophy development.

One litter of second generation 8-day-old SPF pigs that received the dam's colostrum, however, did not show signs of illness postexposure and all developed severe turbinate atrophy 60 days later. Thus, a very important phase in the study of this disease has been reached whereby SPF pigs can now be successfully used to produce the disease experimentally. This will advance the isolation and identification of the causative agent(s).

Further studies on the protective role of the thyroid in hypercalcemic states have been done. The development of AR is independent of dietary calcium and phosphorus. Pig parathyroid hormone has been prepared in large quantities. Also, a radio-immunoassay for thyrocalcitonin has been developed, after being successfully extracted from pigs and purified.

7. Mycotoxins. Studies of acute aflatoxin poisoning in swine were conducted. Crude aflatoxin and purified aflatoxin B_1 were administered to young swine (25-50 lb.) producing acute intoxication and death in 24 to 72 hours. Liver function was markedly altered at 6 hours after intoxication and serum enzymes were markedly elevated after 9 hours. Chromatographs of urine eliminated at 3 to 9 hours showed metabolites of aflatoxin. Pathological changes duplicated those reported for acute natural cases. A combination of chromatographic evidence of the toxin and either clinical pathological or histological evidence of liver damage was considered necessary for diagnosis.

8. Foot-and-mouth disease. A trivalent vaccine combined with oil adjuvant produces a good degree of protection and enduring level of antibody in swine for at least 6 months. The degree of protection is reduced to less than 50 percent at 12 months. Local reactions are evident when swine are inoculated subcutaneously with an oil adjuvant vaccine. However, a subcutaneous inoculation on the posterior surface of the ear provides a satisfactory site for inoculation for antibody response and does not cause carcass blemish.

9. African swine fever. African swine fever virus (ASFV) is disseminated throughout the body by leukocytes after initial invasion of the tonsils. The virus appeared in this site approximately 1 day before the appearance of clinical disease. In detecting ASFV, the fluorescent antibody technique lagged behind the hemadsorption test by 12-24 hours. A hypersensitive reaction thought to be Arthus type was found at the site of inoculation of virulent virus and its appearance was delayed 2 days with attenuated virus. The modified hemadsorption test has proved useful in grouping ASF viruses.

The isolates from 1957 and 1960 disease outbreaks in Portugal were different types, but those isolated, later, in Spain and France were the same as that causing the 1960 outbreak in Portugal. Precipitating and hemadsorption-inhibiting antibodies were produced with Hinde attenuated virus but there is correlation between antibody titers and resistance to subsequent challenge with virulent ASFV. A hypersensitive reaction of an Arthus type was found at the site of inoculation of virulent virus in susceptible pigs. Domestic pigs, in direct contact with wart hogs which were known to harbor the virus, failed to become infected during a 2-year period; they were susceptible to immunity challenge.

North American peccaries and their leukocytes and bone marrow cultures were resistant to infection with several ASF viruses.

Immunosuppressive drugs affected the ASFV course in infected pigs in that the disease course was accelerated, and marked leukopenia was produced. The VERO cell line was more effective than other cell lines for the propagation and adaptation of ASF viruses.

E. Horses

1. Equine infectious anemia. Thirteen farm-raised horses were tested for equine infectious anemia (EIA) virus by horse inoculation with negative results. However, leukocytes of eight of 12 horses examined in a tissue culture system were infected with an apparently nonpathogenic, cytopathic effect producing herpes-like virus. The significance of this virus in horses is unknown, but could be a cause of primary horse leukocyte tissue culture difficulties experienced by most EIA research workers. The attempted adaptation and propagation of four different strains of EIA virus in primary horse leukocyte tissue culture have been unsuccessful to date. The inoculation of a 20-year-old lyophilized culture of EIA virus, Wyoming strain, into a susceptible horse resulted in a positive infection. The horse became a chronic carrier and subinoculation from it produced death in two of three horses. Present investigations include pregnant mare inoculations, and studies of virus attenuation or mutation changes associated with intrauterine disease transmission. Biochemical investigations include electrophoretic studies and analysis of normal and infected horse serums for cholestrol-free fatty acids, phospholipids, triglycerides, and lipoprotein fraction contents.

The transmission of equine infectious anemia virus by the blood worms (Strongylus edentatus and Triodontophorus serratus) was confirmed. The diagnosis of equine infectious anemia using the complement fixation test, siderocyte counts, hepatic biopsies, and serum protein alterations is under investigation.

In cooperative research at the Louisiana State University, Baton Rouge, attempts have failed to transmit EIA by natural breeding to an infected stallion. A colt born of a mare in the latent form of EIA did not develop

the disease.

A standard pool of serum for future work was infective for ponies when 1 ml. of a 10^6 dilution was given. Higher dilutions employed were negative.

Serum protein and lipid changes in acute EIA were demonstrated employing disc electrophoresis and gas chromatography, respectively. Although these changes appear to be remarkable, it is not known if they are unique for EIA. Progress in establishing equine leukocytes in cultures was attained.

At the Texas Agricultural Experiment Station, the nature of the research evolved around characterization of the virus. Studies with horse leukocyte cultures indicate that the virus is reproducing in the lymphocyte. Electron photomicrography indicates that the virus growing in horse leukocytes averages 28 mμ in diameter and is a DNA virus. The abnormal protein has been purified and antibody made. It resembled the hemagglutinin described by Dreguss and Lombard. Hemagglutination was inhibited by the specific antibody as well as blocking the hemagglutin in horse serum.

At the Washington State University, Department of Veterinary Pathology, the leukocyte culture system has been evaluated to determine the various parameters which influence the in vitro propagation of the virus of EIA. The virus can be propagated in these cultures, but the system is somewhat difficult to set up. In addition, the complement fixation test has been evaluated. Antigen concentration was important in determining the antibody titer in infected horses. The antibodies in horses infected with the Wyoming and Texas strains of virus cross-reacted with heterologous and homologous antigen. The disease has been transmitted in three of four horses inoculated with strongyles collected from an animal dying of EIA.

2. African horsesickness. When dogs were fed large amounts of blood and meat from horses infected with African horsesickness (AHS) virulent virus for 9 days, they developed viremia, complement-fixing and neutralizing antibodies, without clinical signs. This finding has provided valuable and firm information regarding the conditions under which the dogs may be infected with AHS. Such information will be valuable in implementation of control measures in the field.

Feeding small amounts of virulent virus in meat or large doses of neurotropic virus in mouse brain developed serological response. Successful experimental infection of adult mice occurred subsequent to administration of the virus intranasally, but not orally, ocularly, or rectally. The information regarding the experimental infection of adult mice with AHS is new and of practical importance to the diagnostician and the virologist working with the vaccine mice-propagated virus strains of AHS.

Using horses, mice, rabbits, and cell cultures, antigens and antiserums for nine virulent and vaccine types of AHS virus were produced. Production and assessment of the reagents provided an opportunity to acquire capabilities

in the diagnosis and typing of AHS virus. Reagents for detection of nine types of AHS viruses by the complement-fixing, virus-neutralizing and infectivity tests were prepared.

F. Fur Animals.

A genetic disease, previously reported only in man, was discovered in mink. It is termed the Ehlers-Danlos syndrome. Histologically, there appears to be a primary connective tissue defect. Pelts from affected animals have no value because the skin is extremely fragile and tears into shreds at pelting. Since the mode of transmission is a dominant characteristic, the disease could spread widely and become a serious problem to an industry having annual pelt sales in excess of 100 million dollars.

The renal glomerular lesions in mink affected by Aleutian disease were examined using light and electron microscopy. The ultrastructural changes were proliferation of mesangial and endothelial cells, increased mesangial matrix, infiltration of inflammatory cells, subendothelial deposition of electron-dense granular material, and accumulation of similar-appearing material in the cytoplasm of mesangial and inflammatory cells. The glomerulopathy is the result of macromolecular stimulation and deposition. The glomerular lesions in Aleutian disease appear to represent an example of a proliferative glomerulopathy which can be initiated by a filterable agent.

Hemivertebrae occurs in mink. This condition has been observed in several color phases and has been studied extensively in the sapphire mutation mink. It is inherited as a simple recessive trait and results from failure of development of one-half of the vertebral body, or failure of fusion of the right and left halves of the vertebral body because of a persistent notochord. The anomaly is similar to the condition in man, but in mink it is particularly dangerous since the animal is extremely supple in its movement. In many mink, the vertebrae are slowly pushed dorsally by the animal's movement and impinge on the spinal cord, resulting in permanent posterior paralysis.

The Chediak-Higashi syndrome (C-HS) is an inherited disease (simple recessive non-sex linked gene) of the membrane-bound organelles of various cells types. Although the condition was known in man, we were the first to report its occurrence in lower animals (mink and cattle). The histological appearance of the inflammatory response observed in C-HS mink, children, and cattle was similar to that evoked by the same agent in non-C-HS individuals. Malignant lymphoma was not present in C-HS individuals. Malignant lymphoma was not present in C-HS individuals of the three species examined. The lesions of Aleutian disease occurred more rapidly in C-HS than in non-C-HS mink, but their histologic appearances were similar.

Mink, man, and cattle with the Chediak-Higashi syndrome are more susceptible to bacterial infections than normal individuals. With the exception of Aleutian disease virus in mink, there is no documented evidence to suggest that C-HS individuals are more prone to virus infections. Studies were conducted to compare the ability of C-HS and non-C-HS individuals to clear phage from the blood and the ability of C-HS and non-C-HS leukocytes to support growth of certain viruses. A test system employing clearance of phage 8 x 174 was investigated and there was no difference in C-HS and non-C-HS mink. The replication and cytopathology of bovine enterovirus (BV-1 strain) in peripheral blood leukocyte cultures obtained from C-HS and non-C-HS cattle were similar. Pseudorabies virus, which does not replicate in normal bovine leukocytes, did not replicate in leukocytes obtained from C-HS cattle. In addition, the extinction curves of this virus were similar when leukocyte cultures obtained from C-HS and non-C-HS cattle were compared.

Thermal inactivation of the infectivity of Neorickettsia helminthoeca is rapid. The rickettsia survived at 37°C. for less than 30 minutes but not for more. Pathogenic rickettsiae were demonstrated in adult flukes, Nanophyetus salmincola, collected from immune animals. This finding might explain maintenance of the rickettsia from one generation of hosts to the next. The presence of metacercariae of Nanophyetus salmincola in smoked salmon for human consumption was demonstrated. Neorickettsia helminthoeca is maintained in trematode vectors and definite vertebrate hosts, but it will not propagate in incubating chicken embryos. The adaptation of the organism in mice would greatly facilitate research studies. Spleen suspensions from infected test animals were inoculated intraperitoneally into mice at 10- to 14-day intervals. The mice developed large lymph nodes and spleens. The rickettsia bodies could be demonstrated in smears of lymph nodes. After 24 passages in mice, the lymph nodes and spleens were enlarged but there was no mortality to serve as an end point. At this passage, the agent had not been successfully adapted to mice.

The capability of Trichinella spiralis transmitting toxoplasma was studied in mice. At appropriate days following administration of trichina larvae, mice were orally inoculated with toxoplasma. Larvae recovered from these mice were passed to other mice; there was no evidence of toxoplasma infection. The larvae recovered from mice following intraperitoneal injections of toxoplasma were also free of the protozoan parasite. It was not possible to transmit Toxoplasma gondii in the mouse with the larvae of T. spiralis when the toxoplasma was introduced intraperitoneally or orally.

Six cases of gray collie syndrome (cyclic neutropenia) in dogs have been reported and the cytopathological and blood protein changes associated with amyloid formation have been examined. Hypergammaglobulinemia occurred early in the disease, whereas in terminal cases with amyloidosis, the gamma globulin was low. In areas of amyloid formation in the spleen, large reticular cells contained amyloid fibrils but endothelial cells did not.

The cooperative research at the University of Wisconsin Department of Veterinary Science resulted in the transmission of encephalopathy to the rhesus monkey killed 33 months after inoculation with a 10 percent encephalopathy brain suspension. No clinical signs of disease were recognized; however, neuropathological examination revealed a polio-encephalopathy similar to that seen in affected mink. Monkey brain and spleen were infectious for mink, with the brain apparently containing a high concentration of the agent.

Two goats inoculated with second passage spleen developed progressive disease with a course characterized by loss of hair, wasting, and incoordination. One animal became debilitated. Pathological examination and tissue inoculation into mink will be made in an attempt to confirm what appears to be an overt transmission of encephalopathy to the caprine species.

Studies on the physical and chemical properties of the mink encephalopathy agent showed it to be less than 50 mμ in diameter, relatively resistant to 10 percent formalin, sensitive to ether, resistant to ultraviolet irradiation, and sensitive to proteolytic digestion with pronase. These results would indicate that the mink agent has physiochemical properties similar to those described for the scrapie agent.

Studies on the transmissibility of mink encephalopathy have indicated that specific neuropathological changes can be induced in diverse species (ferret, monkey, and possibly the goat) after exposure to the encephalopathy agent. The agent also persists for prolonged periods in species (mouse, calf, and cat) in which no pathological alterations were observed.

It should be pointed out that encephalopathy of mink is almost certainly a dead-end infection of mink, acquired by mink only when they are fed infected flesh of the species in which the disease is perpetuated. Evidence that the agent can infect and perhaps persist in several domestic animals and that disease may be induced in the monkey makes it imperative to press research on the epizootiology of this disease. If it is infectious for man as the monkey experiment suggests, and also is present in some livestock species, there may be a real human health hazard as current methods for preparing meat for the table are ineffective in destroying the agent.

In cooperative work at the University of Arkansas Agricultural Experiment Station, the continued study of epizootic abortion in rabbits has added support for its transmissibility. The incidence of abortion was 4.5 percent in the control group, 30.3 percent in the suspect buck group and 16.7 percent in the Mima challenge group. In addition to losses from obvious abortion, the litter size and 24-hour survival of newborn rabbits were reduced in the suspect buck group.

Mucoid enteritis has been investigated further and severe outbreaks occurred involving adult animals. Attempts were made to transmit the condition by injecting whole blood or plasma into young rabbits. However, the apparent

effect was to reduce the symptoms of the disease in the inoculated group as compared to controls.

Rabbit growers submitted 119 rabbits for diagnosis of disease problems. The most commonly encountered diseases were those involving the digestive and reproductive systems.

Tests designed to determine the most effective treatment for outbreaks of several diseases were conducted. Excellent results were obtained using Neomycin and Neomycin plus Terramycin in an outbreak of Escherichia coli enteritis. Tetracyclines were apparently beneficial in outbreaks of mucoid enteritis but no treatment attempted seemed beneficial in abortion outbreaks.

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None

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None

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None

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None

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None

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None

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None

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CONTROL OF INTERNAL PARASITES OF LIVESTOCK AND POULTRY
(RPA 212)

USDA and Cooperative Program

Location of Intramural Work	Commodity	Scientist Man-years FY 1968
New Mexico (Las Cruces)	Cattle	0.2
Alabama (Auburn)	Cattle	2.5
Georgia (Experiment)	Cattle	1.0
Maryland (Beltsville)	Cattle	13.1
New Mexico (Albuquerque)	Cattle	0.5
Maryland (Beltsville)	Sheep and goats	6.9
New Mexico (Las Cruces)	Sheep and goats	2.8
Alabama (Auburn)	Sheep and goats	0.5
New Mexico (Albuquerque)	Sheep and goats	2.5
Maryland (Beltsville)	Poultry	7.2
Georgia (Tifton)	Swine	1.0
Maryland (Beltsville)	Swine	1.5
Maryland (Beltsville)	Horses	3.3
	Total	43.0

Intramural program is supplemented by extramural support representing (a) 2.2 SMY's at State Agricultural Experiment Stations and Universities and (b) P.L. 480 funds in four countries.

Problems and Objectives

Internal parasites, such as various kinds of worms, flukes, and coccidia, cause losses in all parts of the country and in all seasons. In general, warmth, moisture, and shade favor parasites. About 300 kinds are of economic importance in the United States, and will cause losses estimated at $650 million annually by 1980, at present rates. Severe infestations of parasites may cause heavy direct losses to the livestock producer, but internal parasites generally are unseen, their effects are not apparent, and the loss to the public from inefficient production is hidden. Losses include mortality, reduced yield, condemnation of meat, feed wastage, and cost of drugs. Even for the parasites that have been the subject of considerable research, treatment or control measures are far from adequate.

Research objectives include studies of:

1. Biotic relationships in parasitism.
2. Parasite control by biological methods and management practices.
3. New and improved safe chemical means of combating parasites.
4. More effective means of diagnosing parasitic infestation.
5. Microbial agents that utilize parasitic life cycles for transmission.

Progress - USDA and Cooperative Programs

A. Cattle

1. Helminthiasis. Data are being gathered to analyze statistically the effect of gastrointestinal nematodes on the ability of the host to digest food. A new technique, using finely divided polyethylene powder, Microtene, is being used.

Wastelage was investigated to determine its epizootiological implications. This product, made by combining feedlot manure and ground coastal Bermudagrass hay, is a low-moisture silage having high nutritive value and excellent palatability. As no larvae were recovered from the silage, it was concluded that Wastelage was not a potential carrier of parasitic nematode infections.

In general, results obtained during the second of a 3-year study showed that an increase in the stocking rate resulted in an increase in the number of nematode parasites recovered at necropsy, and in a reduction in the weight gains made by beef cattle. Results obtained from the three groups of cattle with different levels of parasitic infections were different from those obtained the previous year, probably because of the prolonged drought experienced that spring, when all tester steers were removed from the pastures. The 3rd-year test was concluded and the number

of worms present and the species represented from each of the steers are being determined at the present time. However, an increase in the stocking rate reduced the weight gains of the steers, and the steers from the group with low level of parasitism had greater weight gains than those from the other two groups. An exploratory experiment was also concluded to study the epizootiological and economical implications associated with the feeding of a grain feed supplement to cattle on pastures. The parasites recovered from the slaughtered steers are being counted and identified at present.

Work is in progress on cross-immunity studies with four species of the nematode genus, Trichostrongylus--T. affinis, T. axei, T. calcaratus, and T. colubriformis in rabbits. All of these parasites will mature in rabbits, although two of them, T. axei and T. colubriformis, are ruminant parasites. Since they parasitize three different regions of the rabbit's gastrointestinal tract, it is believed that useful information on the development of immunity to gastrointestinal parasites can be acquired from this research.

Studies indicated that an infection with Trichostrongylus affinis in rabbits can persist for as long as a year, and that the length of patency was not influenced by the number of larvae administered.

Previous work showed that infective larvae of T. affinis are found in the cecum after 24 hours. Passage of the parasite through the intestinal tract will be investigated by means of infection and subsequent slaughter at intervals of 2, 4, 6, 8, 10, etc., hours until the larvae arrive in the cecum.

Under a cooperative project at the University of California, School of Veterinary Medicine, Davis, California, three anthelmintic trials were conducted on clinically parasitized cattle. The fact that no benefits accrued from therapy tends to discredit the view that any number of parasites is harmful.

Temperature and moisture levels affected development and survival of cattle nematode larvae on pasture. The lesser stomach worm, Ostertagia ostertagi, required a temperature of 55° to 70°F. and the cooperids and nodular worms, 60° to 70°F., for good development. One to 4 inches of rain per month were required for all three genera. Maximal recovery of infective larvae was obtained at an average monthly temperature of 70°F. Monthly rainfall required was 1.77 to 5.42 inches. The initial, peak, and final recoveries of infective larvae from feces and from herbage occurred on the average 4, 6, and 14 and 5, 11, and 16 weeks after deposition of the feces, respectively.

The smallest number of worms developed in calves from a challenge infection of 150,000 infective larvae of several species of gastrointestinal nematode parasites administered 32 days after a comparable initial dose. This finding may indicate that the primary infection was most effective in preventing establishment of new infections acquired at this time. The initial population of the lesser stomach worm, O. ostertagi, was not affected by any of the challenges, which were administered 10, 20, 32, and 46 days after initial infection, but challenges at 32 days and 46 days may have caused partial loss of adult Cooperia.

Infections with the stomach hairworm of cattle, T. axei, appeared to interfere with the development of other gastrointestinal nematode species in this host. Fewer worms developed in a challenged calf than in the similarly infected challenge control calf, and development of the worms was also retarded in the former. In calves necropsied 2 weeks after the challenge dose was administered, 99 percent of the Cooperia in the challenged calf were in the fourth stage, while only 6 percent were in this stage of development in the challenge control. This finding is significant in that the primary infection consisted of a parasite belonging to an entirely different genus from those in the challenge dose.

Administration of Azium, an anti-inflammatory agent, did not alter the natural resistance of calves to the sheep nodular worm, Oesophagostomum columbianum, nor did it reduce the severe tissue reaction evoked by the invasive larvae.

The swine nodular worm, O. dentatum, produced lesions in the cecum and greater omentum of calves, but failed to develop beyond the parasitic third stage.

Withholding feed from guinea pigs before inoculation with the ruminant parasite, T. colubriformis, resulted in enhanced infections. This appeared to have a physiological rather than a mechanical basis, as none of the inoculum was lost by spillage or regurgitation.

Nematodiasis is dependent on the continuous presence of worms in the gastrointestinal tract. Anthelmintic clearance of T. colubriformis from guinea pigs given known lethal doses of larvae prevented death.

Two species of ruminant parasites, T. axei and T. colubriformis, can be readily established in hamsters, providing an additional experimental model for studies on pathogenesis in nematode infections.

Previous trials indicated that lambs might be produced parasite-free twice yearly by breeding Dorset ewes in October or January, isolating the lambs at birth, and raising them on artificial diets, in strict quarantine and sanitation. In this year's breeding trials, most of the Dorset ewes bred

in October or January produced lambs. Pregnancies and multiple births were greatest in October breedings. Each time, some lambs were isolated at birth and others were left with ewes 8 to 24 hours and then isolated. Comparisons were made with lambs raised by ewes. The lambs isolated at birth and those left with ewes 8 to 24 hours before isolation, remained parasite-free with no evidence of other diseases. Lambs raised with ewes readily acquired parasites. The artificially-reared lambs grew practically as well as those naturally reared, and fewer died. The artificial diets fed the isolated lambs appeared satisfactory. The findings indicate that for practical production of parasite-free lambs, isolation of the young animals may be deferred for up to 24 hours after birth with reasonable degrees of safety, if strict isolation and sanitary procedures are employed thereafter.

It was determined that chickens might be substituted for lambs in some aspects of studies aimed at improving procedures for disease-free lamb production.

Two formulations of l-tetramisole were tested for anthelmintic activity in 30 cattle under field conditions. The drench (8 mg./kg. body weight) and the bolus (5.4 mg./kg. body weight) were 94-100 percent effective against the adult forms of Haemonchus placei, Cooperia spp., T. colubriformis, O. ostertagi, O. radiatum, and Bunostomum phlebotomum. Effectiveness against T. axei was 81 and 85 percent, and against larval forms was 67 and 87 percent, respectively, with the two formulations. There was no evidence of intoxication.

The efficacy of Maretin was tested as an anthelmintic in cattle. Critical tests using four steers indicated that, when administered as a drench at the rate of 50 mg./kg. of body weight, Maretin was highly effective against H. placei, C. punctata, C. oncophora, and T. colubriformis. It was somewhat less effective against T. axei and O. ostertagi. No signs of intoxication occurred.

Critical tests showed that a dose level of 2 mg./kg. of body weight of Baymix Crumbles, given for 6 consecutive days, was highly effective against H. placei, C. punctata, C. oncophora, and T. colubriformis, and to a lesser degree against O. ostertagi. No signs of intoxication were apparent.

Blood samples were taken from a calf before and after infection with larvae of C. oncophora. The serums were collected twice a week beginning 1 week before infection and continuing through the course of infection with the parasite. Infective third-stage larvae homogenized by means of a French press were used as antigen source. Ouchterlony and latex agglutination tests were used to detect the presence of antibody in the calf serums. All Ouchterlony tests were uniformly negative; however, one serum sample

taken 7 days after infection gave a positive test with a latex agglutination trial, indicating the presence of a slight level of antibody against larval antigen.

Chromosomes of the C. oncophora-surnabada complex were demonstrated for the first time. Experiments designed to isolate C. surnabada from C. oncophora have been concluded. Both types of worms frequently are found parasitizing the same host and although adult males can be differentiated morphologically, the adult females are morphologically indistinct so far as is known.

Two helminth-free calves were infected with larvae of both the C. oncophora and the C. surnabada types. One calf was killed 8 days after infection when the parasites were in the parasitic fourth-stage of development. Female helminths of both types were isolated from the males in the intestinal contents and injected into a previously prepared helminth-free calf via a duodenal fistula. The second infected calf was killed approximately 1 week later when the worms were in the parasitic fifth-stage of development. Only male C. surnabada helminths isolated from the intestinal contents of this calf were used in infecting a previously fistulated calf. After the onset of patency, eggs passed in the feces of the fistulated calf were allowed to develop in cultures and the resulting infective third-stage larvae were used to infect another helminth-free calf. After allowing sufficient time for the parasites to reach maturity, the calf was killed and the parasites examined.

This experiment was repeated two more times, once using the original fistulated calf after it had been dewormed of parasites using various anthelmintics and once using another newly fistulated calf.

In all three cases, both types of male worms were found in the intestinal contents of calves harboring the progeny of unions with C. surnabada males and females of C. oncophora and C. surnabada, giving strong evidence that C. oncophora and C. surnabada are members of the same species.

Oxygen uptake studies were also conducted on infective third-stage larvae of C. oncophora at various temperatures. The larvae were obtained from Baermannized fecal cultures. The larvae samples used in this study were homogeneous, although the larvae themselves were not of a uniform age because of the protracted period of time necessary to accumulate sufficient numbers of cleaned larvae. Respiration of the larvae was measured using the standard Warburg technique. Approximately 41,800 infective third-stage larvae were used per flask. Oxygen consumption in microliters/hour/41,800 infective third-stage larvae ranged between 1.02 at 10°C. and 12.25 at 30°C.

A research contract was initiated at the University of Wisconsin to study variations in pathogenicity among several isolates of Haemonchus contortus. It appears that the more pathogenic populations had a greater fecundity.

2. Coccidiosis. Microisolation of pure cultures of Eimeria bovis (and also of E. ahsata of sheep) of cattle was continued by using a laser microscope. Attenuation of the destruction by the laser beam was produced by reducing the joules of energy input into a ruby laser. A reduction to 175 or 125 joules enabled a reduction from complete destruction of oocysts to only cracked oocyst walls or, at lower energy, only a formation of one or two bubbles of steam about 2 to 10 μ in diameter inside the oocyst. Penetration of a blue dye into the cracked walls served as indicators of the amount of destruction and enabled identification of irradiated oocysts. Damaged oocysts were prevented from sporulating (becoming infective) while adjacent oocysts that were not struck sporulated as usual. As the microscope "safety interlock" is not safe and will not prevent dangerous specular reflections in a laser room, a device was constructed that completely removed the radiation hazard and was accident-proof.

Cytochemical investigations of acid and alkaline phosphatases, carboxylic ester hydrolases (CEH), "leucine" aminopeptidase, phosphorylase, and succinate, lactate, and glucose-6-phosphate dehydrogenases have been conducted on the endogenous stages of E. stiedae. Acid and alkaline phosphatases have been found in all of the endogenous stages (schizonts, merozoites, sexual stages, and unsporulated oocysts), and their detailed localization has been described. Although all endogenous stages contained CEH, not all of them possessed all of the subgroups of these enzymes as these subgroups are defined according to their behavior toward organic phosphate (diethyl-p-nitrophenyl phosphate) and p-chloromercuribenzoate.

The merozoites within the schizonts, the female gametocytes, and at least some oocysts contained an apparently very small amount of, and/or a lowly active or inaccessible form of, an enzyme or enzymes which will liberate naphthol from L-leucyl-4-methoxy-b-naphythylamide. In accordance with common histochemical practice, this enzyme(s) would be considered as "leucine" aminopeptidase.

An attempt has been made to characterize partially the phosphorylase system in E. stiedae; but at the present time, the results are both confusing and conflicting, and conclusions have not been drawn. It appears that both phosphorylase 1a and 1b, and perhaps, "branching enzyme" are present in at least some stages of the coccidia at certain times. However, possibly, the cytochemical results are influenced by the metabolic situation prevailing in the cell at the moment it was frozen.

All of the dehydrogenases concerned were ultimately demonstrated in each endogenous stage of E. stiedae. This finding would suggest that these stages possess at least portions of the pentose shunt, the Emden-Meyerhof pathway, and the tricarboxylic acid cycle.

Experiments to determine whether Ethoxyquin (1,2 dihydro-6 ethoxy-2,2,4 trimethylquinoline) a common feed additive, was related to abortion or stillbirths in rabbits were concluded. Data do not indicate that Ethoxyquin in the diet of rabbits is related to abortion or stillbirths in rabbits.

Oxygen uptake studies were conducted on unsporulated oocysts of E. stiedae at various temperatures. Unsporulated oocysts were obtained from the livers of rabbits killed approximately 23 days after infection with 1,000,000 sporulated oocysts. Approximately 90 percent of the oocysts had the capacity to complete sporulation. Respiration of oocysts was measured using the standard Warburg technique. Approximately 2.3 billion oocysts were used per flask. Oxygen consumption in microliters/hour/2.3 billion oocysts ranged between 2.22 at 5°C. and 24.99 at 35°C.

Seven additional experimentally-infected cows were treated during the current reporting period. One was given oral dimetridazole, 50 mg./kg., daily for 5 days. The others were given single intravenous injections of the drug at a level of 50 mg./kg. At six post-treatment examinations during a 4-month period, trichomonads were not recovered from the cow treated orally, nor from five of the six cows that received the chemical intravenously. Trichomonads were recovered from one of the intravenously-treated animals at the fourth examination made approximately 3 months after treatment. This animal then was given an intravenous injection of 100 mg./kg., but the infection persisted. Tritrichomonas foetus infections have now been eradicated from nine of 11 dimetridazole-treated cows.

Under a P.L. 480 research project at the Hebrew University, School of Pharmacy, Jerusalem, Israel, hemolysins have been isolated from Ochromonas malhamensis and O. danica and found to be toxic. Further characterization studies of these toxins revealed that they are saponins. Incorporation of rhodamine dye into culture medium may serve to differentiate this group of organisms.

3. Anaplasmosis. Preliminary studies with an alpha-dithiosemicarbazone (Contrapar) for its efficacy in destroying the bovine carrier state of Anaplasma marginale (anaplasmosis) infection showed that oral or parenteral administration of the drug can eliminate the infection. This indicates that chemicals other than tetracycline inhibit the causal disease agent.

Further standardization and evaluation studies of a rapid-card-agglutination test for bovine anaplasmosis showed that correlation with complement fixation test results is greatest when heparinized plasma is used, and when the card test is conducted in 4 minutes. Card antigen, with a high degree of uniformity in sensitivity, has been prepared from four different donor animals. The test has been applied to more than 800 animals, and an overall agreement with complement fixation of 87 percent was obtained.

A cooperative project was recently begun at the University of Missouri, School of Veterinary Medicine, to determine if there are exoerythrocytic stages of Anaplasma marginale.

4. External parasites. Identifications were made on 194 lots of specimens (1 protozoan, 11 cestodes, 9 trematodes, 162 nematodes, 3 acanthocephalans, 6 arthropods, and 2 miscellaneous lots). The specimens and their hosts were diverse and mainly of veterinary importance; e.g., thread-necked strongyle, Nematodirus spathiger, found in a horse for the first time; numerous nematodes of various wild ruminants; hundreds of ear mites, Otodectes cynotis, found on a wolverine in Alaska; lice, Echinophthirius horridus, from seals in Alaska and California; and a first instar of a cattle grub, Hypoderma sp., removed from a bovine heart. Immature Cysticercus bovis found dead by meat inspectors in beef carcasses of the same origin suggest the possibility that these immature cestodes may have been killed by a systemic insecticide used to control cattle grubs.

B. Sheep and Goats

1. Helminthiasis. In experimental work, fourth-stage larvae of the large stomach worm (Haemonchus contortus) were recovered from lambs as long as 33 weeks after infection. This finding helps explain the epidemiology of pasture infections in that the inhibited larvae obtained the previous fall may mature the following spring with a resultant contamination of the pasture.

Third- and fourth-stage larvae of the large stomach worm failed to immunize lambs against subsequent challenge with that worm. Larvae from the initial infection were removed after 7 days with heavy doses of thiabendazole before challenge 20 days later.

A suspected early date of whipworm infection for lambs was confirmed experimentally. The lambs became infected within a few days of birth, possibly ingesting worm eggs on the udders of the ewes.

A study was conducted to determine the carry-over of sheep parasites from 1 year to the next on a contaminated pasture. A gradual increase in parasites was noted. Continual exposure to Nematodirus larvae, however, appeared to bring about the development of resistance to further infection

after 3 months. Most of the worms recovered appeared to have been carried over from the last grazing season, which ended in December.

Lambs on pasture in Nebraska apparently developed an immunity to Nematodirus spp. after the first season's exposure, and 5-year-old or older ewes had about half the numbers of nematode eggs in their feces as younger ewes. Native Nebraska Corriedale ewes had much higher egg counts than those of Targhee, Navajo, or Suffolk breeds.

Recent collections of liver flukes (Fasciola hepatica) in eastern Arizona showed at least a 29 percent incidence in 52 head of cattle from six ranches and a 27 percent incidence in 15 head of sheep on two ranches. While this incidence is considered high, it is somewhat lower than that observed in previous years in sheep and cattle in northern New Mexico and southern Colorado.

Several drugs effectively eliminate adult liver flukes but most are ineffective against immature flukes except at dosage levels toxic to sheep. In recent investigations using both naturally- and experimentally-infected ewes, Tremerad, Bayer 9015A, and sulfoxide of bithionol eliminated more than 95 percent of the flukes 6-weeks-old and older with little, if any, toxicity. Since most of the damage in acute fascioliasis is caused by immature flukes migrating through the liver tissue, these drugs are potentially of great value to sheepmen in fluke-infested areas.

Two snails, Lymnaea bulimoides and Fossaria modicella, are principal transmitting agents of the common liver fluke in the Southwest; these snails have been propagated in our laboratory and infected experimentally with the larval stages of F. hepatica. The importance of knowledge concerning the biology of these forms can be readily appreciated. There is marked age resistance in these snails to the miracidia of F. hepatica; thus, in nature, control measures should be directed at the young snails.

Rambouillet and Corriedale lambs responded similarly to immunization procedures involving the large stomach worm. Comparisons were made by using an antelope strain of the parasite to immunize lambs of the two breeds. The immunizing infections were removed with an anthelmintic, and principals and controls were challenged with a sheep strain of the parasite. As evidenced by degree of anemia and intensity of infection, there was no significant difference in the way the two breeds reacted to challenge.

Despite reports in the literature to the contrary, we have been unsuccessful in demonstrating that a functional immunity to haemonchosis can be produced by injecting larvae intraperitoneally.

Previous studies on parasites of elk in New Mexico showed the presence of the winter tick, the fringed tapeworm, and lungworms. Additional records for this year include the roundworms, Elaeophora schneideri, Ostertagia ostertagi, Trichostrongylus axei, and T. colubriformis. These are new records for New Mexico. Apparently O. ostertagi and T. colubriformis have not previously been reported in elk anywhere. Since all of the parasites listed above have been reported in domestic ruminants, the elk should be considered a potential source of infections.

Recent work in our laboratory showed that T. colubriformis occurred in 20 of 25 jackrabbits collected on sheep range. The incidence in javelina was much lower than in jackrabbits. These observations suggest that jackrabbits should be investigated as potentially important reservoirs of infection for sheep.

In trials with lambs, thiabendazole given orally at 50 mg./kg. of body weight was more efficacious against one isolate of the large stomach worm, Haemonchus contortus, than against another isolate (AH-2) of the same parasite. Differences in percentage efficacy were observed against both mature and immature parasites in each of two trials, and all calculated efficacies were lower than those commonly reported with thiabendazole at 50 mg./kg. Because of the relatively small number of lambs employed in these trials, however, further replications are needed to establish whether differences in the response of the two strains to thiabendazole are statistically significant.

A new character has been found useful to identify the six species of thread-necked strongyles of domestic sheep in the United States. The number, size, and arrangement of bosses (blister-like swellings) on the bursal lobes of the males varied among species. This new character, with others recently found, should prevent the confusion which has prevailed in taxonomic and experimental work relating to the speciation of Nematodirus abnormalis, N. davtiani, N. filicollis, N. helvetianus, N. oiratianus, and N. spathiger.

Morphologic and histochemical studies are in progress on the intestinal nematode, Cooperia punctata, of sheep and cattle. The fine structures of male worms have been defined by conventional methods. These structures are now being redetermined in body sections and their chemical composition ascertained.

Abdominal nematodes, Setaria, from horses, mules, donkeys, cattle, bison, antelope, deer, and moose in the United States and Canada are being studied. Three species have been found and their correct nomenclature, hosts, geographic distribution, and characters to easily differentiate them, are being determined.

A description was prepared of a new medium stomach nematode of deer with a key for the identification of the 9 species of stomach worms of deer in North America. The new species is known to parasitize Odocoileus virginianus in Georgia, Louisiana, Pennsylvania, and Ontario. It has not been found in domestic ruminants. A description was also prepared of a new species of physaloperid nematode found among worms collected in a lizard in Africa.

At the Kentucky Agricultural Experiment Station, the cooperative research was concerned with the evaluation of anthelmintic drugs. The anthelmintic dl-tetramisole administered orally at 15 mg./kg. provided a broad spectrum of activity against the major nematodes in the lambs in a controlled test. While the 10 mg./kg. dose was very effective on mature nematodes, it was less effective than the 15 mg./kg. dose on immature nematodes.

Naphthalophos at 25 and 50 mg./kg. levels was highly effective against a strain of H. contortus that was isolated previously and was tolerant to 50 mg./kg. doses of thiabendazole. Numbers of second and third instars of Oestrus ovis were not sufficient for evaluation, while data on the first instars did not definitely establish removal activity.

Under a P.L. 480 grant to the University of Warsaw, Warsaw, Poland, the research effort was concerned with the immunological response of sheep and goats to strongyloidiasis. Three immunizing doses of larvae were necessary to produce sufficient immunity. The best immunizing dose proved to be 50,000 larvae of the rabbit strain, which was obtained after at least 40 passages of the sheep strain through rabbits. Also, the X-ray-irradiated larvae produced better immunity than normal larvae.

Under a P.L. 480 grant to the Institute for the Application of Nuclear Energy in Agriculture, Veterinary Medicine and Forestry, Belgrade, Yugoslavia, the research study was concerned with the irradiated and normal metacercariae of Fasciola hepatica at various intervals after infection in mice.

Culture of the snail, Lymnaea truncatula, intermediate host of the liver fluke, was established under laboratory conditions and successful production of the fluke larval stages was achieved. The cercariae were irradiated at levels from 2 to 4 kiloroentgens (kr) using an X-ray source. Experiments in albino mice demonstrated the reduced pathogenicity of irradiated forms as compared to controls. Structural changes, resulting from the radiation treatment, were observed in the juvenile flukes recovered subsequently from the livers. Histomorphologically, radiation caused destruction and elimination of the epithelial cells lining the ceca and suppression of the genital organs. On the basis of these results, an X-ray dose of 3 kr was selected for further experiments.

Infection rates in mice with normal cercariae, or cysts, ranged from 3 to 34 percent compared with 0 to 26.8 percent resulting from the infections with irradiated cysts.

The histopathology in the liver was similar for mice receiving normal or irradiated fluke cysts 1 week after infection. However, at the end of the second and, particularly, the third week of infection with irradiated cysts, there was a tendency for encapsulation of the focuses of damage caused by juvenile flukes. Proliferation of mesenchymal cells and the appearance of connective tissue indicated repair of the lesions. Irradiation of the cysts had mostly a lethal effect on the subsequently-derived flukes. They died later than 3 weeks postinfection.

Histologically, flukes appeared to undergo lysis. In contrast to this, normal flukes showed no changes in morphology, and progressive destruction of the liver parenchyma, in addition to a general absence of lymphoid and mesenchymal cell reaction, was observed to the end of a 6-week experiment.

In an 8-week experiment, mice survived a repeated infection, 4 weeks apart, with 100 irradiated cysts.

The immune response to infection was determined serologically, by the hemagglutination and complement fixation techniques, and by the miracidial test. Circulating antibodies were detected using the complement fixation technique and miracidial test; however, they were not detected by hemagglutination.

The research results are only preliminary, but when completed with further investigation data, they could provide much needed information on the liver fluke. It would also be applicable to other trematodes of the liver, and, in fact, trematodes in general. The results would be used for advanced studies on the immunity of helminths and, possibly, in developing a program of immunoprophylactic control of fascioliasis.

Under a similar grant to the Institute of Preventive Veterinary Medicine, Belgrade, Yugoslavia, the research effort dealt with the pathogenesis and prophylaxis of anaerobic diseases of sheep associated with parasitism by liver flukes. The results showed that in 64 percent of bacteriologically-examined sheep with an established infection, anaerobic microorganisms have been isolated from the liver (Clostridium oedematiens B, Cl. perfringens A, Cl. perfringens C, and Cl. septicum). No pathogenic microorganisms were isolated from 36 percent of the carcasses. Of the established microorganisms, the largest number was Cl. oedematiens B (44 percent), the constant inhabitant of the digestive tract of sheep. This is also proved by the fact that it was found with other clostridial microorganisms in 13 percent of sheep affected with Cl. perfringens C, and in 7 percent of sheep affected with Cl. perfringens A.

The results of our experimental investigations showed a close relationship existing between the quantity of anaerobic infections and the incidence of helminthic infestations. This indicates that parasites not only carry germs from the digestive tract into the liver, but also damage liver tissue, creating favorable conditions for the multiplication of all anaerobic germs in the liver.

A P.L. 480 grant was executed during the reporting year with the Sri Venkateswara University, Tirupati, India, for the study of certain snails which are intermediate hosts of important parasites such as liver flukes. The objectives of this project are to compare the habits, environment, and metabolism of infected and noninfected snails to provide information for snail and parasite control.

2. Coccidiosis. Additional studies were made on tissues of lambs that had been infected with oocysts of Eimeria intricata, the species that produces the largest oocysts in the digestive tract of sheep. Gametogenesis occurs in the small intestine, cecum, and colon, with the majority of the gametocytes being found in the lower half of the small intestine, instead of being restricted to the jejunum as was reported last year. Many stages were located in the cecum. Only two were seen as far down as the midcolon. The parasites were limited to the epithelial cells lining bottoms of the glandular pits. The average size of 20 macrogametocytes was 24.5 μ by 38.5 μ. The average size of 20 microgametocytes was 44.3 μ by 96.4 μ. There was a tendency for mature microgametes to migrate into adjoining cells. Some of what appeared to be single, large microgametocytes may have been made up of more than one with the microgametes coalescing. Sporulated oocysts of E. ahsata were cracked mechanically to release sporocysts. These were concentrated in great numbers by centrifugation and were imbedded in Maraglas or Vestopal and polymerized for future examination of sporozoites by electron microscopy.

3. External parasites. The only treatment for head grubs of sheep (Oestrus ovis L.) now recommended by the USDA is a drench of a 21 percent Ruelene suspension. This material is expensive and hard to obtain in small quantities as required by owners of small farm flocks, or for 4-H projects. In an effort to find some readily available, commercial product which would be inexpensive and easy to use on a small number of sheep, two aerosol bombs were tried as nasal sprays on 16 sheep. Fifteen untreated sheep from the same flock were used as controls. Some toxicity was encountered following the use of one of the bombs, and neither proved universally effective. The best control was obtained with 8-10 g. of the more toxic material delivered in one 4-second burst in each nostril.

Some progress and new technological gadgetry was gained in our attempts to rear O. ovis in vitro. Although many difficulties still exist, the problem does not appear beyond solution.

Various members of a closely confined laboratory flock of approximately 50 sheep of mixed breeds and ages have harbored Psorergates ovis, the Australian itch mite, since 1963. During 1967, live mites were recovered from only two sheep; in 1968, both of these were declared negative, while three other subjects were positive. Individual hosts appear to retain infestations for an average of 2 years. Clinical signs of acariasis, in our recent observations, are limited to a mild pruritus. These factors, in addition to the extremely small size of the parasite, help to explain the elusiveness of psorergatic acariasis in the USA.

Experiences with Psorergates bos, during a 5-year period, were similar to those with Ps. ovis. Enormous numbers of skin scrapings from a once heavily infested bull, during 1967 and 1968, suggested that psorergatic infestations on individual bovine animals survive for 2 years or less; as in sheep, spontaneous recovery may be anticipated. Natural transfer of mites to cattle of all ages, in close confinement, has not been accomplished.

Three discrete and highly pathogenic strains of Ps. ovis have been maintained for several years on six isolated groups of sheep (total of 120). In 3 of the subgroups heavily infested subjects are replaced periodically with clean, normal sheep, while the hosts in the remaining subgroups are not replaced. Studies, based on clinical responses to the parasites, reveal that in nature, particularly in a geographical region where summer latency of mite populations is pronounced, 1) protective immunological responses of host to parasite exist only on a temporary, cellular level, and 2) strain pathogenicity or virulence does not diminish as a result of host association during a 3-year period.

Tests with several candidate acaricides for the control of sheep scabies (Ps. ovis) demonstrated that ciodrin (Shell), a broad spectrum insecticide, registered for livestock use against lice, flies, and other arthropod parasites, continues to show unusual promise as a sheep psoropticide when administered as a dip. Another product, Prolate, or Imidan (Stauffer) also continues to prove biologically effective against scabies of sheep in dip form, but currently presents formulation difficulties.

Spinose ear ticks (Otobius megnini) are common and destructive parasites, widely distributed among livestock of the arid and semiarid regions of western USA. Tests with candidate acaricides suggest that biologically effective drugs, when administered to cattle in jelly or paste form inside the external auditory canal and around the base of the ear, provide longer residual activity against the return of seed ticks than other methods of administration.

Exhaustive studies, conducted in cooperation with the Hides and Leather Laboratory, EURDD, ARS, concerned with the pathogenicity of the sheep tick or ked (Melophagus ovinus) have been rewarding. Damaged sheepskin is the

primary injury inflicted by this parasite. Among other losses are diminished wool production and fiber damage, carcass downgrading, reduced feed conversion efficiency, and possibly reduced carcass dressing percentage. These investigations are expected to come to early fruition, and are designed to assist a lethargic industry.

Tissues of domestic and wild animals in the Southwest were examined for the presence of demodectic mites. Mites were found in the eyelids of 10 percent of the cattle, 33 percent of the goats, and 80 percent of the horses examined. None were found in eyelids of sheep, deer, elk, or antelope. Tissues other than eyelids were found positive for mites in two of three fox specimens examined.

Immunological investigations of demodecosis of livestock remain inconclusive. Antigens prepared from bacteria- and lipid-free demodectic mites from goat skin lesions produced no reaction in immunized rabbits on agar gel precipitation tests.

The seasonal incidence of clinical demodecosis in dry and lactating dairy cows is being investigated; infestations within herds as low as 1 percent during winter months, and as high as 60 percent during May, have been reported.

A study of tissues from necropsied dogs in a 1,000-animal colony revealed that demodectic mites are present on or in the skin of animals showing no clinical evidence of demodecosis; skin samples from 15 percent of the mature dogs, and 18 percent of newborn puppies contained mites.

C. Poultry

1. Coccidiosis. Sporozoites of Eimeria meleagrimitis and E. tenella, liberated from oocysts by grinding and excysted with trypsin-bile, were frozen and stored in liquid nitrogen vapor for 6 months without loss of viability. When inoculated into cell cultures of bovine embryonic kidney, both species developed to mature schizonts. Frozen sporozoites inoculated into birds produced oocysts and lesions similar to those in birds inoculated with freshly excysted sporozoites. Storage of coccidia by this method may save the laboratory considerable time, effort, and expense in maintaining stock cultures of coccidia.

Eimeria meleagrimitis and E. adenoides developed through one complete asexual generation in cell cultures of embryonic turkey intestine and embryonic bovine kidney when maintained at alternating intervals at 43 and 40.5°C. In the bovine kidney cultures, schizonts were produced that were bigger and with more merozoites than previously described from experimental infections in the host. Evidence is being gathered that indicated that inability to complete the cycle in vitro is due to inability of bovine cells to completely adapt to the temperature of the turkey.

By using cell culture, it was determined that decreased infectivity of older oocyst cultures is not due to inability to excyst, but rather to 1) death of sporozoites within sporocysts during storage, and 2) inability of sporozoites to survive and develop after entering cells.

The only carbohydrate demonstrated within sporozoites growing in cell culture, within oocysts, during and after excystation, and in the normal host was a homogeneous polysaccharide of glucose (amylopectin). Two enzymes, chymotrypsin-like and trypsin-like, were found in homogenates of oocysts of both E. meleagrimitis and E. tenella.

A strain of the chicken coccidian, E. tenella, that developed a tolerance for Novostat, as a result of serial propagation in chickens fed mash containing the coccidiostat, proved cross resistant to two other commercial coccidiostats, Unistat and zoalene.

Nine strains of E. tenella, each strain resistant to a different commercial coccidiostat, showed no cross resistance to two newly developed avian coccidiostats, buquinolate and metichlorpindol.

Eimeria meleagrimitis, a coccidian parasite of turkeys, was developed in chickens when given daily intramuscular injections of the immunosuppressant drug, dexamethasone, a synthetic corticosteroid.

Over 20 attempts to process E. meleagrimitis indicated that problems in fixing, infiltrating, embedding, and sectioning may be due to difficulties in separating oocyst wall particles from sporozoites before the processing.

2. Blackhead. Histomonas meleagridis cultivated in vitro for 8 consecutive years practically lost its ability to enter, and multiply in, the host's tissues, but remained antigenically similar to fresh parasites, as determined by the reciprocal fluorescent antibody test. The test always clearly distinguished this species, regardless of source, from H. wenrichi, a closely related species.

Ring-necked pheasants can be important in the spread of histomonads and cecal worms to chickens and turkeys, as well as to other game birds. The cecal worm thrives almost as well in pheasants as in chickens, and the pheasant is almost as resistant to blackhead as most breeds of chickens. Chukar partridges proved so susceptible to histomoniasis as to be considered unimportant in the spread of the disease. Bobwhite quail, poor hosts for the two parasites, probably are unimportant in their spread. The findings regarding pheasants may explain sources of some blackhead outbreaks of unknown origin that have occurred in turkeys.

Histomonas of cecal origin proved almost 100 times as infective to turkeys as did parasites from liver lesions. In nature, only histomonads from the cecum are involved in transmission. However, parasites from liver lesions are sometimes used for basic experimentation. When used, their infectivity limitations should be considered. Factors responsible for these limitations probably include nutritional requirements of the parasites.

A strain of the poultry blackhead organism, H. meleagridis, which was propagated for 6 months in birds given suboptimal levels of dimetridazole, did not develop a tolerance for the chemical.

In cooperative work at the Texas Agricultural Experiment Station, College Station, cell cultures of H. meleagridis were contaminated with yeast-like organisms. Similar yeasts were readily isolated in cell culture from clinical cases of histomoniasis. The significance of the agents has not been determined. Additional attempts to transmit H. meleagridis using the darkling beetle (Alphitobius diaperinus) failed.

The cooperative research at the University of Minnesota, College of Veterinary Medicine, was concerned with the in vitro cultures of H. meleagridis. Cultures have been maintained in vitro for 41 and 43 days in Lund's medium with normal and modified concentrations of Panmede. In cultures of Histomonas, two distinct groups of bacteria seemed to be present; specific isolations were Escherichia coli and Bacillus cereus. It appears that successful long-term cultivation of H. meleagridis depends upon establishing a delicately qualitative and/or quantitative synergistic relationship of bacterial associates. Studies are now in progress to determine the nature of the immunological response of experimentally-infected turkeys to H. meleagridis. Work will be carried out to observe the nature and response of host tissues to the parasite and of the parasite to the host.

Efforts to maintain the English strain of Heterakis gallinarum in chickens has been continued. Massive inoculations of young chickens failed to produce infections of more than 131 nematodes per bird. Adult male chickens appear to lose their infections with H. gallinarum 3 to 4 months postinfection.

3. Other parasites of poultry. l-Tetramisole, a drug given by mouth at 30 mg./kg. body weight, was 100 percent effective against adult and immature Ascaridia dissimilis, 98 percent effective against adult H. gallinarum, and 89 percent effective against adult Capillaria obsignata in turkeys. This is the first drug proved to be efficacious against Capillaria in turkeys. Parbendazole given by mouth at 30 mg./kg. was 97 percent effective against Heterakis, but was relatively ineffective against Ascaridia (17%) and Capillaria (27%). l-Tetramisole given at 0.05 percent in feed for 1 day was highly efficacious against Ascaridia, removing all of the adult and 91 percent of the immature worms; parbendazole at 0.05 percent in feed was 100 percent effective against adult Ascaridia, but removed only 45 percent of the immature worms. Both drugs were well tolerated.

Styrid [styrylpyridinium = 2-(p-chlorostyryl)-1-methylpyridinium chloride] given to dogs at 5 mg./kg. body weight/day for 10 days in the feed was highly effective against larval hookworms; and when given at 20 mg./kg. orally as a single dose, it was 99 percent effective against adult hookworms (Ancylostoma caninum).

The anthelmintics, phenothiazine, Ruelene, thiabendazole, Haloxon, dl-tetramisole and l-tetramisole were tested in the Mongolian gerbil-Trichostrongylus spp. host-parasite system to determine its utility as a primary screen for the discovery of potential anthelmintics. This system, which employs a small laboratory animal infected with two species of pathogenic nematodes of domestic animals, proved satisfactory for the intended purpose, as only one anthelmintic (phenothiazine) of six tested failed to show activity.

Poultry gapeworm (Syngamus trachea) perienteric fluid hemoglobin has an average molecular weight of 38,397 as compared to 64,906 for host turkey hemoglobin. Peptide maps of worm and host hemoglobins reveal many common peptides. However, the parasite hemoglobin contains four peptides not found in turkey hemoglobin while the turkey hemoglobin contains four peptides not found in the worm hemoglobin. In addition, the worm hemoglobin has no trypsin-resistant core found in many vertebrate hemoglobins, including that of the turkey. Previous work showed other differences between the parasite and host hemoglobins. Differences may indicate how gapeworms form their hemoglobin and if they use host hemoglobin for this purpose. Barium antimonyl tartrate, a remedy for gapeworms, kills the worms grown in in vitro cultures at a level of 0.005 g./100 ml. Equivalent amounts of barium tartrate, antimony tartrate, or potassium antimonyl tartrate are equally toxic. The combination of barium and antimony is not needed for toxicity.

Under P.L. 480, a grant has been awarded to the Mar Ivanios College, Trivandrum, India, to investigate the resistance potential of domestic fowl to infection by the tapeworm Raillietina tetragona. Resistance will be measured in terms of growth and conversion of feed under a variety of husbandry practices.

D. Swine

1. Helminths. Six pairs of Hampshire pigs were repeatedly exposed to 100,000 infective larvae of Strongyloides ransomi. Five immunized pairs and one control pair were later challenged with one million infective larvae of S. ransomi.

A negative correlation was noted between number of exposures and worm egg counts, as well as number of exposures and number of worms at necropsy. Female pigs had higher egg counts and more worms at necropsy than barrow pigs.

Increases in eosinophils and basophils were associated with S. ransomi infection. A decrease in rate of daily gain occurred following the first exposure only to infective larvae.

Adding 65 g./ton of pyrantel hydrochloride to a complete pig ration was beneficial to 20-lb. parasitized pigs. Twenty-four pigs fed the chemical for 42 consecutive days gained an average of 0.08 lb. more per day than did 24 unmedicated controls. The treated pigs had 49 percent fewer intestinal threadworms (Strongyloides ransomi) and 65 percent fewer ascarids (Ascaris suum) than did the controls when both groups were necropsied at an average weight of 200 lb.

Incorporation of 0.02 percent thiabendazole in a complete ration fed to 22-lb. parasitized pigs did not increase their rate of gain over that of unmedicated pigs. When necropsied at an average weight of 75 lb., the 20 medicated pigs had 46 percent fewer intestinal threadworms (S. ransomi) than did the 20 control pigs.

Embryonation of A. suum eggs was prevented following exposure for 3 days to thiabendazole at a concentration of 1:1000.

Nematodes of swine which use dung beetles as intermediate hosts are of economic importance because of losses incurred from unthriftiness, morbidity, or death of parasitized animals. Larvae of the thick stomach worm of swine, Physocephalus sexalatus were found in 65 percent of Phanaeus vindex beetles examined during spring and early summer.

Flight activity of P. vindex begins an hour after sunrise with a temperature of at least 57°F. Activity decreases at temperatures above 92°F. and ends an hour before sunset. Phanaeus igneus requires a temperature of 62 to 87°F. for activity. Phanaeus igneus is adapted to a wooded environment whereas P. vindex is more often found in open areas. Both species of beetles were more strongly attracted to swine feces than to the feces of other domestic and wild animals.

Juvenile and adult swine kidney worms (Stephanurus dentatus) have respiration rates (microliter O2/mg. N/hr. uptake) in the following order: adult male > juvenile male > juvenile female > adult female. Study of worm intestines showed 1) peroxidase activity is associated with host white blood cells, 2) large amounts of sulfide present nonspecifically reduce indicators in tests for steroid dehydrogenase activity, 3) cholesterol, cholesterol esters, and other unidentified steroids are present, and 4) progesterone, corticosterone, cortisone, estradiol, and testosterone appear to be lacking. Cholesterol content varies but apparently is present in juvenile and adult forms in the following order: adult females > juvenile females > adult males > juvenile males. These findings are background studies on steroid biosyntheses and transformations which may lead to discovery of compounds analagous to insect juvenile and moulting hormones.

Continuous flow electrophoresis separates complex mixtures of antigens and enzymes present in chromatographic fractions of excretory gland extract. DEAE-cellulose anion exchange and Sephadex gel filtration chromatographic columns yield only partial separations. Isolation of the antigens may improve means of diagnosis and immunization. Granules in the excretory gland can be isolated by sucrose gradient centrifugation. These are ruptured at pH 8 and liberate, among other constituents, a chymotrypsin-like enzyme. This indicates a secretory function for excretory glands. Histochemical and electron microscopic studies of the glands show a huge nucleus, the granules, and many of the organelles common to other cells. The granules are PAS-positive and are negative for glycogen, lipids, and nucleic acids.

Suspensions of infective eggs or larvae stored over liquid nitrogen for 7 to 82 days were thawed and examined for viability (motility). Up to 12 percent of Stephanurus dentatus regained motility. However, only 1 percent of Oesophagostomum columbianum and less than 0.1 percent of Ascaris lumbricoides were viable.

Stephanurus dentatus maintained in vitro in cell-free mediums, or tissue culture, produced antigens continuously for 3 to 4 months. Antigens stored at -25 to -85°C. for 18 months gave precipitin reactions comparable to fresh material. Thus, direct qualitative comparisons could be made of the influence of longevity, substrate, and sex on antigen production.

Heretofore, it was known that swine spontaneously eliminated A. lumbricoides about 21 days after infection. This has been characterized as an immune phenomenon triggered by antigens released at 4th molt. Elimination of an earlier intestinal stage occurred 10 days after infection at the time of 3rd molt. The dual elimination suggests that the substance (antigen) released at both ecdyses is not stage specific, but instead is characteristic of the molting process.

Microorganisms were isolated from two types of cuticular lesions of A. lumbricoides. From type 1: coliforms (Escherichia-Aerobacter) and enteric streptococci; from type 2: Candida sp., coliforms, and enterococci. Organisms found not associated with lesions were: coliforms, streptococci, and Saccharomyces sp. (perivisceral fluid); Gram-positive rods (intestine); and yeast-like forms (uterus).

Microorganisms found in ureteral cysts and in Stephanurus dentatus contained therein were compared. Coliforms, nonenteric streptococci, and Bacillus sp. were found both in worms and cyst fluid. Saccharomyces sp. and Alternaria sp. were isolated only from worms. Staphylococcus sp., Cladosporium sp., and enteric streptococci were found only in cyst fluid.

l-Tetramisole given to swine at 15 mg./kg. for 1 day in the feed was 99 percent effective against the nodular worm, Oesophagostomum dentatum. Haloxon given at 50 mg./kg. for 1 day in the feed was 75 percent effective against this parasite.

In the cooperative research at the North Carolina Agricultural Experiment Station, a new anthelmintic agent, pyrantel tartrate, was found to be extremely efficient in preventing the migration of Ascaris through the liver of swine. Work was undertaken to evaluate the effectiveness against migration stages of the swine kidney worm.

Thirty pigs were placed on the growing ration containing 65 g./ton of pyrantel tartrate at weaning. An equal number were fed the same ration but without the drug. They were confined in pastures that had been contaminated by sows with patent infections of swine kidney worm and Ascaris.

Six weeks after initiation of trial, 5 pigs from each group were killed and the liver lesions scored. The pigs on ration containing pyrantel tartrate had no Ascaris lesions and an average of 5 kidney worm lesions. Those on nonmedicated feed had livers that would be condemned on a kill floor because of Ascaris and kidney worm lesions. There was an average of 29 kidney worm lesions in untreated swine.

Pigs reared under a modified specific pathogen-free program were placed on feed containing 130 g./ton of pyrantel tartrate for 3 days and then given 2,500 kidney worm larvae orally. Similar pigs were given nonmedicated feed and a challenge infection of larvae.

These animals were killed and livers examined for evidence of kidney worm lesions. The only lesions indicative of kidney worm were in untreated pigs.

The cooperative study at the University of Nebraska, showed that parbendazole is highly effective against Ascaris suum and Trichuris suis in swine. Parbendazole offers a broad spectrum of activity, wide margin of safety, and ease of administration which should make this new compound one of major importance in swine herd therapy for the control on internal parasites.

A check on ascariasis in finishing feeding pigs, as measured by fecal egg counts made on composite samples taken at 140 days, showed that rate of growth is not significantly affected by worm load. However, mortality for Ascaris-infected pigs was 2 percent higher.

Further studies on the aminopeptidases of A. suum present in the intestinal extracts revealed that the major portion of the aminopeptidase extracted and partially purified from A. suum was previously obtained only with a low specific activity (0.3 units/ mg. protein). However, treatment with trypsin-chymotrypsin resulted in a preparation that was then purified to have a specific activity of 2.75 units/mg. protein with little or no loss in enzymatic activity. Characteristics of this aminopeptidase were similar to that previously purified from the same source. Autolysis of crude intestinal homogenates also resulted in increasing amounts with time of a component, which corresponded to the characteristics for the purified aminopeptidases.

Two aminopeptidases which hydrolyze l-leucine-B-naphthylamide were partially purified from uterine extracts of adult A. suum. One aminopeptidase was purified 29 times by ammonium sulfate precipitation, fractionation on carboxymethylcellulose, and Sephadex G-100. The other aminopeptidase constituted only a small portion of the total aminopeptidase activity and was prepared by DEAE-cellulose fractionation of ammonium sulfate precipitated fraction.

Both enzymes were maximally activated by cobaltous ion but vary somewhat in the activation by other divalent metal ions. Other characteristics serve to distinguish these aminopeptidases and also to differentiate them from the previously purified aminopeptidase from intestinal extracts of A. suum.

2. Trichinellosis. Initial gas-liquid chromatographic tests disclosed that the rare, volatile, fatty acid, n-valeric, was present in Trichinella spiralis larvae, larva-containing cysts, and cyst-containing pig muscle. Later tests showed it to be present in other muscle systems, organs, and blood serum from trichinous pigs. The content was from 0.0005 to 0.001 mg.%. Tissues from nontrichinous pigs have none or only trace amounts. N-valeric acid content above trace amounts may be diagnostic of T. spiralis infection.

An antigen made from dried, sonicated, trichina cysts suspended in buffered saline (pH 7.2) elicited a skin reaction characterized by an indurated area, 12 mm. in diameter, surrounding a small red center, 1 mm. in diameter, when injected intradermally in the vicinity of the xiphoid process of trichinous pigs. This reaction occurred as early as 7 days after experimental infection with 500 trichinae/pound of body weight. Fifteen- and 21-day infections gave rise to intense reactions. All reactions persisted for 24 hours. Additional work is needed to determine the smallest infection detectable with the antigen and its adaptability to field conditions.

Arrangements have been completed with the Animal Health Division, ARS, the Technical Services Division (MI), C&MS, and Livestock Conservation, Inc., to conduct a cooperative pilot project to evaluate the pooled sample digestion technique for detecting trichinae in hog carcasses in a commercial meat packing establishment.

A second series of tests with a more sensitive pH meter and more heavily infected trichinous pigs failed to confirm the finding reported last year that trichinous pork may have a relatively higher pH than non-trichinous pork.

Initial gas-liquid chromatographic tests showed T. spiralis larvae, larva-containing cysts, and cyst-containing striated muscles contain acetic, n-valeric, and hexanoic acids. Later tests showed esophagus, stomach, kidney, liver, and blood serum from trichinous pigs contained these acids. Propionic, isobutyric, n-butyric, and isovaleric acids are also usually present; pivallic, isocaproic, and heptanoic acids are rarely present. Acetic acid content is high: 0.001-0.01 mg.%, while that of the other acids is lower. N-valeric acid content is from 0.0005-0.001 mg.% or more. Tissues from normal and ascarid-infected pigs may also have a high acetic acid content but usually have none or only trace amounts of n-valeric and the other acids. The presence of n-valeric acid in greater than trace amounts may be diagnostic for trichinella infections. N-valeric acid in normal tissues may come from the trace amounts found in certain lots of the hog's food. Perivisceral fluid of adult ascarids contains acetic, propionic, isobutyric, n-butyric, isovaleric, and isocaproic acids. Gravid S. dentatus females contain acetic, propionic, and isovaleric acids.

In Poland, the Agricultural University College, Wroclaw, the P.L. 480 grant was used to simplify and improve present methods of diagnosis, to evaluate factors of the host-parasite relationship, and to clarify the action of drugs useful in the treatment of trichinellosis.

The methods employed were mostly autoradiographic and histochemical. Using J^{131} or P^{32}, it has been demonstrated that both isotopes introduced into the host body readily reach altered tissues of the host and the parasite. The cyst formed about the host does not act as a barrier to the passage of substances from the parasite to the host. Characteristic accumulation of large amounts of the isotope in certain morphological elements at various intervals after infection justifies some conclusions about the function of the elements, larval nutritional mechanism, and function of its internal organs.

A similar study was done of histochemical changes in the host. Ten respiratory enzymes and phosphorylase activity were studied to check the previous biochemical observations on respiratory disturbances, metabolic pattern of infected muscles, and localization of processes involved.

Proof that leukergy appears early in both animals and human beings infected with trichinellosis has practical implications. The test, easy to perform, when coupled with other nonspecific procedures, can be used in the clinical diagnosis of trichinellosis.

E. Horses

1. Equine piroplasmosis. Using indirect fluorescent antibody technique, a procedure was developed for detecting carrier horses infected with Babesia caballi; there were no cross reactions with B. equi. Weakly positive reactions were difficult to detect.

The intraerythrocytic development of each equine Babesia was studied, using time-lapse cinematography of unstained blood preparations and light microscopy of fixed, stained blood films. Babesia caballi developed from a round anaplasmoid body into a ring-like structure which developed into two piriform parasites. Frequently an anaplasmoid body was extruded from the blunt end after the piriform shape was attained. Babesia equi seemed to develop from an anaplasmoid body in the same manner as B. caballi, except that the chromatin material organized into four or five masses on the periphery of the ring-like structure. These masses became nuclei of the four or five piriform bodies characteristic of this species.

Babesia caballi was successfully stored for 1,040 days, and B. equi for 603 days, in liquid nitrogen. Glycerol was used as a cryoprotective agent. Both species, upon injection into equine animals, produced typical signs of disease. The antigenic characteristics of each, as determined by the complement fixation test using antiserums of known titer and specificity, were unchanged.

The development of B. caballi in Dermacentor nitens was traced in smear preparations and histologic sections of infected ticks. Most of the parasites in equine erythrocytes ingested by the adult ticks apparently were destroyed. Small spherical bodies 4-6 μ in diameter were the first developmental stages of B. caballi observed in the gut contents of ticks. These spherical bodies apparently gave rise to clavate (club-shaped) bodies, 10-14 μ long by 4-6 μ wide. The latter developed into large round bodies, 12-16 μ in diameter, that segmented into vermicular-shaped parasites, about 8-12 μ long by 2-4 μ wide. Some penetrated the gut wall, and some invaded other cells of the tick.

In the cells of the Malpighian tubules, hemolymph, and ovaries, the vermicular parasites underwent a secondary cycle of multiple fission, forming vermicules similar to those occurring earlier in the gut. Vermicules that invaded the ova underwent a similar multiple fission cycle during the larval stage of the tick.

Vermicules from the multiple fission cycle that occurred during the period of larval feeding invaded the salivary glands. A multiple fission cycle of increase within these glands resulted in large numbers of small, oval, and piriform parasites, 2.5-3 μ, maximum dimension. These parasites became mixed with the salivary secretions, and presumably are the forms injected into the horse by the nymphs as they feed. The small oval and piriform parasites therefore appear to be the infective stage for the horse.

Apparently B. equi-infected red blood cells are best processed by pelleting them in a 1 percent agar solution and fixing them in a buffer of pH 7.4 of 280 to 290 milliosmols.

In the cooperative research at the Kentucky Agricultural Experiment Station, attempts have been made to establish B. caballi in species other than the equine animal.

A carrier state was created in a pony with B. caballi and its blood was inoculated into two splenectomized ponies that subsequently died from the disease. A high percentage of red blood cell infection occurred in both animals before death. One had 0.82 percent of the erythrocytes infected while the other had 1.8 percent infected. Whole blood was collected from these two ponies in the terminal stages of the disease and injected into 138 laboratory animals (mice, hamsters, rabbits, guinea pigs, cats, and dogs) in varying dosages and routes. Another trial using blood from a splenectomized pony with a 1.7 percent infected erythrocyte count was conducted using 38 animals. All of the above species of laboratory animals plus sheep were used. On the basis of microhematocrit determinations and stained blood smears, there was no evidence that infections of B. caballi were established in any of the animals in these trials.

Attempts to cultivate B. caballi on tissue cultures of primary horse kidney cell monolayer and equine bone marrow resulted in no cytopathic effects. The inoculated tissue culture mediums suspended in saline and injected in a splenectomized pony failed to reproduce the disease. A serial sacrifice experiment involving nine splenectomized ponies was conducted to study the histopathology of piroplasmosis. These animals were inoculated with an equal amount of blood from a pony with 0.95 percent infected erythrocytes. Individual animals were necropsied on days 3 through 12. Of the nine animals, seven were euthanatized and two died from the infection. The main changes from the disease occurred in the lung, liver, heart, and kidney. The primary finding was an extensive mononuclear cell infiltration, together with changes associated with blood destruction. One of the basic findings in this research is that B. caballi appears to be a host-specific parasite. An infection could not be established in any of the commonly used laboratory animals. Tissue culture attempts were not successful in propagating the organism. The histopathological findings

indicate that heart damage is the probable cause of death in acute cases, rather than anemia.

The cooperative work at the University of Florida was concerned with the evaluation of drugs and their application in control of B. equi infections in horses. Whereas Diapram and Phenamidine had been effective in eliminating the carrier state in B. caballi-infected horses, neither drug was as effective against B. equi infections.

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A. Cattle

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PROTECT LIVESTOCK AND POULTRY FROM TOXIC CHEMICALS, POISONOUS PLANTS, AND OTHER HAZARDS (RPA 213)

USDA and Cooperative Program

Location of Intramural Work	Commodity	Scientist Man-years FY 1968
Utah (Logan)	Cattle	2.5
Texas (Kerrville)	Cattle	4.5
Texas (College Station)	Cattle	2.7
Utah (Logan)	Sheep and goats	2.5
Texas (Kerrville)	Sheep and goats	0.5
Texas (College Station)	Sheep and goats	0.3
	Total	13.0

Intramural program is not supplemented by extramural support.

Problems and Objectives

Livestock and poultry may suffer losses in productivity from atmospheric pollutants and pesticide residues remaining on crops used for animal food. Poisonous plants can cause heavy losses, particularly when pasture or range feed supplies are short or at seasons of the year when these plants are not discriminated against by the grazing animal. Predators cause heavy damages to sheep and turkeys.

Research objectives include studies of:

1. Specific sites and mechanisms of poisonings.
2. Toxicology of pesticides and other chemicals used directly on, or occurring in, the environment of livestock and poultry.
3. The metabolic fate of pesticides and other chemicals likely to be ingested by livestock and poultry.
4. Management practices that minimize the use of pesticides and other chemicals that leave toxic residues.
5. Prevention or alleviation of the effects of toxic plants.

Progress - USDA and Cooperative Programs

A. Cattle

1. Poisonous plants. Congenital anomalies in lambs induced by maternal ingestion of Veratrum californicum, and crooked calf disease induced by maternal ingestion of Lupinus caudatus and L. sericeus, were prevented after animals were given vitamin A added to mineral supplement. These results must be carefully studied and the experiment repeated next year before any significance can be shown that vitamin A supplement is an alleviator in preventing congenital anomalies induced by maternal ingestion of these poisonous plants. If a feed supplement can be found to prevent these congenital deformities, over $1,000,000 will be saved annually for the livestockmen.

Larkspur poisoning (Delphinium occidentale) has been prevented for 2 consecutive years in a herd of 77 cattle on a heavily infested larkspur range. All animals were given a mineral supplement to stimulate the growth of rumen flora. In previous years, three to seven animals have died annually from larkspur poisoning on this range.

Four experimental pastures have been established in the Caribou National Forest for scientifically-controlled experimental studies on the prevention of larkspur poisoning in cattle. The studies will be done cooperatively by Caribou National Forest Service, Forest Service Research Laboratory, Idaho Beef Council, Malad Idaho Cattlemen's Association, and the Poisonous

Plant Research Laboratory of the U.S. Department of Agriculture, Agricultural Research Service, Animal Disease and Parasite Research Division.

Studies were carried out on 70 Angus cows that, under field conditions, had been poisoned on Astragalus lentiginosis. Only 28 cows gave birth to viable calves. The remaining cows aborted and two died. Five cows were brought to the laboratory for studies which are currently in progress. The studies include a pathological examination of livestock poisoned on locoweed.

Atrazine, a commonly-used herbicide for controlling undesirable weeds and grasses, although incriminated in reports from several livestockmen, caused no adverse effects on cattle and sheep when forage sprayed with moderate and high levels of the herbicide were fed to the animals for 30 days. No subclinical effects were found on a detailed analysis of blood constituents, enzymes, and histopathological tissue sections.

Similar studies are underway using another triazine herbicide, prometone.

Tordon, a picolinic acid derivative, caused no clinical symptoms in sheep and cattle fed the substance with alfalfa hay at 1 and 2 lb./acre. Previously, it did not cause subclinical effects when fed on alfalfa to sheep at the same levels.

2. Pesticides and other chemicals. Water and mud samples from the watershed (at 4 points below the laboratory) were analyzed for the presence of 29 different pesticides, representing every category utilized in research activities. Utilizing gas chromatography, no detectable amounts of any of the chemical compounds were found. A similar study, measuring possible atmospheric contamination from incineration, was carried out with negative results, also. The inaugural study, involving a fungicidal compound, will be made, utilizing a liquid scintillator on a triple-labeled radioisotope product. This will greatly reduce the time required in the study of metabolism and residue of pesticides in livestock. As the result of suspected toxicity of a calf nursing a cow exposed to a plant insecticide, laboratory procedures were established to determine the probable metabolites in the milk.

Analyses were made on a series of dipping vat samples to detect the concentration of insecticide in the vat at intervals during the dipping process, thereby determining selective takeout by the animal's wool or hair. These data, with parasite kill, are used to evaluate (1) the insecticide, and (2) the formulation utilized. Metabolism of a chlorophenoxy compound has been studied in vitro and in vivo with extraction procedures for two probable metabolites established. Gas chromatographic sensitivity is greatly increased by reacting these metabolites to produce trimethysilyl derivatives.

Tissue sections were examined microscopically from animals and birds that were exposed to agricultural chemicals and died, or were euthanatized. Microscopic changes span a range of lesions, varying from compound to compound, dose, and duration. The hepatotoxic and nephrotoxic effects of some compounds were somewhat consistently found, and, in general, reflect histopathological changes common to a number of chemical poisons. One or more sets of specimens from subjects exposed to five organic herbicidal, three fungicidal, nine insecticidal, four chemosterilant, and six miscellaneous compounds were studied by 3,831 routine histopathological, 155 special, and 707 blood smear-stained sections. Repeated exposures of sheep, at chronic toxic dosage levels, to diallate, a herbicidal compound (resulting in loss of wool), are being investigated from its histopathological and etiological aspects. Various blood parameters are being measured, including plasma copper levels, as well as feed utilization and fluctuations in body temperature. After 5 months, there have been no noteworthy alterations. A study involving the relationship of fetal erythrocytes and hemaglobin to the adult type following plant insecticide toxicosis in calves is being continued with more emphasis on the normal or control calves.

Swine poisoned with large single oral doses of carbaryl had signs of parasympathetic nerve stimulation, including salivation, tremors, vomiting, depression, anorexia, cyanosis, and, ultimately, death. The signs could be controlled with injections of atropine.

When swine were given lower doses of carbaryl for 8 to 12 weeks, entirely different signs were seen. The first evidence of poisoning was a relaxation of the flexor tendons of the hind limbs, followed by incoordination, ataxia, exaggerated gait in the hind limbs, partial paralysis, and death. This syndrome could be corrected by repeated daily administration of a diuretic (Esidrex) even though the carbaryl dosing continued. Swine not given the diuretic died.

Three pigs paralyzed by continuous daily dosing with carbaryl were studied for changes in sciatic nerve impulse conductance and velocity. The impulse velocity in vivo was essentially unchanged between control and poisoned swine, but velocity in vitro was slower in nerves from poisoned swine.

Chronic carbaryl poisoning signs resemble those seen in deficiencies of thiamine or pyridoxine. Neither vitamin was of any effect in relieving carbaryl poisoning.

A pig paralyzed by repeated carbaryl dosing was then maintained on the carbaryl while monosodium glutamate was given in the feed daily. After 5 days, the pig was able to walk, and after 8 days, was much improved. Biochemical changes during poisoning are being studied.

B. Sheep and Goats

1. Poisonous plants. Feeding a diet containing halogeton increased water consumption. Restricting water intake to sheep receiving a diet containing halogeton caused a marked decrease in feed intake. This decrease was not observed in sheep on a nonhalogeton-containing diet. When water was supplied every other day to sheep receiving a diet containing halogeton, feed consumption decreased to near zero while feed consumption to sheep fed a diet containing no halogeton was not markedly affected. Sheep fed halogeton over a period of approximately a month and then fed a lethal dose (based on what is lethal to a sheep not preconditioned to halogeton) were not affected.

These results indicate that, under natural conditions on desert ranges, there is probably a marked relationship between water intake and halogeton consumption. It further suggests that excessive consumption of halogeton, resulting in sheep mortality, can be prevented by supplying adequate water.

Pregnant ewes were fed Veratrum californicum (false hellebore) on 2 successive days during the 10th to the 41st day of gestation. Cyclopia, previously reported, was induced in the offspring of the ewes fed on the 14th and 15th days of pregnancy. Two other types of teratogenic conditions were produced, both at specific periods of gestation. A "seal-type" deformity characterized by pelvic fusion and posterior deviation of the hind limbs was produced in offspring of ewes fed on the 16th to 19th day of gestation. Shortened hind limbs that were caused by a marked decrease in the length of the tibia and fibula were produced in the offspring of ewes fed on the 26th and 27th days of pregnancy. These 2 periods of insult have not been reported previously in the deformities induced by V. californicum.

A review of the records of previous feeding trials in sheep involving V. californicum, as well as data collected this past year, indicated that in some sheep the conceptus did not remain viable, in utero, throughout the entire gestation period. Many embryos died during the early period of gestation, resulting in recurrence of estrus in the ewe at a later date. Embryonic deaths coupled with the deformed fetuses that were caused by pregnant ewes ingesting V. californicum, represent a serious economic loss to the sheepmen of the intermountain west.

The structure was elucidated for the teratogenic steroidal alkaloid, cyclopamine, from V. californicum responsible for cyclopian and related central nervous system malformations in sheep. Data established the structural identity as 11-deoxojervine. Preliminary investigation of alkaloid X suggests its structure as 3-glucosyl-11-deoxojervine. The single preparation of veratrosine previously found to have cycloptic activity was significantly contaminated with alkaloid X. Thus, cyclopian

teratogenic activity appears to be restricted to the three structurally similar, steroidal alkaloids, jervine, 11-deoxojervine, and 3-glucosyl-11-deoxojervine.

Cyclopamine proved to be highly acid-labile. In vitro incubation in conditions of acidity and temperature found in single stomach animals resulted in very rapid dehydration and oxide bridge cleavage to veratramine, a compound devoid of cyclopian activity. Thus, the apparent ability to produce cyclopian malformations only in ruminants, and not in single stomach animals, may be a function not of rumen organisms, but the high acid lability of cyclopamine.

The feeding of potato and tomato vines and fruit failed to produce cyclopian effects. Thus, steroidal alkaloids from these sources are not active if dose levels are high enough.

Aminoacetonitrile, the compound postulated to be involved in loco plant-produced malformations in sheep, was fed to over 50 ewes at various periods of gestation. Typical loco-like malformations were produced at nearly all periods of gestation except during about the first 25 or last 25 days. Compounds fed in an effort to reverse this lathyrogenic effect failed to do so in either aminoacetonitrile- or loco plant-fed animals. They also failed to reverse the neurological signs of locoism. A polar phase from a 30 percent ethanol extract of loco plant produced both malformations and other signs and lesions of loco plant-fed animals.

Aminoacetonitrile and an extract from lupine plants both caused malformed calves similar to the lupine plant-induced crooked calf disease.

It has been shown during this past year that the time of insult to a developing fetus in a ewe poisoned on locoweed is chronologically very nonspecific. Abortions can occur in locoed sheep during all stages of gestation.

Aminopterin, a folic acid inhibitor, causes congenital deformities in rats very similar to those occurring in locoweed poisoning in sheep and cattle. Aminopterin was injected subcutaneously into 20 bred ewes during various periods of gestation. Congenital deformities and abortions very similar to those occurring in locoweed poisoning were observed.

In Skull Valley, Utah, 6,280 sheep developed a mysterious illness characterized by muscular incoordination, scoliosis of the cervical, thoracic and lumbar spines, distinct droop of the head, nasal discharge, and frequent micturation. Erythrocytic cholinesterase activity was severely depressed. Nearly 2,000 sheep died within 2 days after onset of clinical signs, and 2,500 died or were destroyed during the following 2 weeks.

The U.S. Army's Dugway Proving Ground is adjacent to Skull Valley, and it was reported that the day before the sheep became ill, agents designed for chemical warfare were tested. These agents were similar to "nerve gas" developed near the end of World War II.

Investigation into the deaths of the sheep disclosed that the cause of death was not related to known poisonous plants, but it did indicate that the disease was probably forage-related.

Forage was gathered in the area where the sheep had become ill and fed to sheep. Clinical signs, identical to those observed in the affected sheep in Skull Valley, were produced in the experimental sheep. Examination of the erythrocytic cholinesterase activity revealed that the activity was severely depressed.

2. Pesticides and other chemicals. Acute and chronic toxicity trials were conducted in cattle, calves, sheep, goats, and chickens to as many as seven organophosphorous plant insecticidal compounds. Dosage levels ranged from the peracute to maximum safe amounts as administered by oral capsules, mixed with the feed ration, or sprayed on vegetation in a restricted enclosure. No ill effects to repeated exposures of these insecticides could be detected in lactating cows, or nursing calves.

Oral and dermal toxicities of 45 other insecticides were investigated in cattle and calves in cooperation with Entomology Research Division. The oral toxic effects of five fungicidal compounds were determined to cattle, sheep, and chickens with feed utilization and conversion rates measured. Toxicity trials, generally supplementing previously collected data, were carried out with 38 herbicidal compounds. Experiments on calves and sheep were inaugurated with repellant and nematocide compounds.

A study was carried out with sheep orally-exposed to propazine, indicating that restricted water intake had no effect on its toxicity. The acute and chronic effects, as well as the residue, of an organophosphorous insecticide has been determined to 4- and 6-week-old turkeys. These data were supplemented by feed utilization and conversion measurements. Further trials will be conducted at 20 to 24 weeks of age. A radiotelemetric system for measuring temperature, heart rate, and other physiological parameters as an aid to toxicity diagnosis in animals is now operational.

From a preliminary investigation, it was found that two herbicidal compounds, an organic chlorophenoxy and a thiocarbamate, resulted in an alteration of the Ca-Mg ratio in plasma and tissue of exposed cattle and sheep. Further study utilizing technical materials and commercial formulations indicated no difference in the activity of the parathyroids

and thyroids of sheep with the chlorophenoxy herbicide. There was a change in toxic reaction between the technical and commercial formulation of the thiocarbamate to sheep; however, there was no change in the mineral ratio of cattle from the technical material. Data collected were subjected to biometrical analysis. Lead determinations on paint utilized on fences surrounding sheep-holding pens revealed that it presented no hazard to animals. Lead and copper determinations were made of various tissue and fluid samples from an experimental cow to establish the cause of death. Studies have been initiated in the analysis of the exotoxin of Bacillus thuringiensis Berliner, a possible cause of reproduction failure in another ARS Research Division's cattle herd.

Carbaryl was fed at 25 mg./kg. daily to ewes from 2 weeks before conception to parturition. All ewes delivered perfectly normal lambs, indicating there will be no teratological effects in sheep as a result of agricultural use of carbaryl, and that the danger to other ruminants (cattle and goats) may also be quite low.

Acute poisoning was induced in sheep with ammonium chloride, ammonium sulfate, and a mixture of ammonium chloride, ammonium carbonate, and ammonium phosphate. Toxicity was due to the ammonium ion since signs of poisoning were the same regardless of the anion.

In addition to the lowering of blood pH and greatly elevated blood sugar levels reported last year, serum potassium and nonprotein nitrogen levels were markedly increased during ammonium ion poisoning. The total amino acids of the nonprotein nitrogen fraction of the blood were also increased. The effects upon blood sugar levels are due to either an adrenergic response, or an interference with sugar metabolism, or both. The elevated blood potassium levels appear to be due to massive cellular breakdown during poisoning. Terminal increase in amino acids indicates an overloading of the urea cycle.

Extensive histological changes were observed, including generalized hemorrhages from the vascular beds of the body with marked hemorrhages in the thymus. There was severe active congestion of the lungs, accompanied by hemorrhages, atelectasis, and severe damage to the alveolar cells. There was severe tubular nephritis. The central nervous system showed severe congestion accompanied by numerous small hemorrhages and by neuronal degeneration.

In sheep poisoned by toxaphene, there are significant increases in blood sugar and a significant lowering of blood amino acids. No further conclusions can be made at this time.

Publications - USDA and Cooperative Program

A. Cattle

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B. Sheep and Goats

1. Poisonous plants.

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